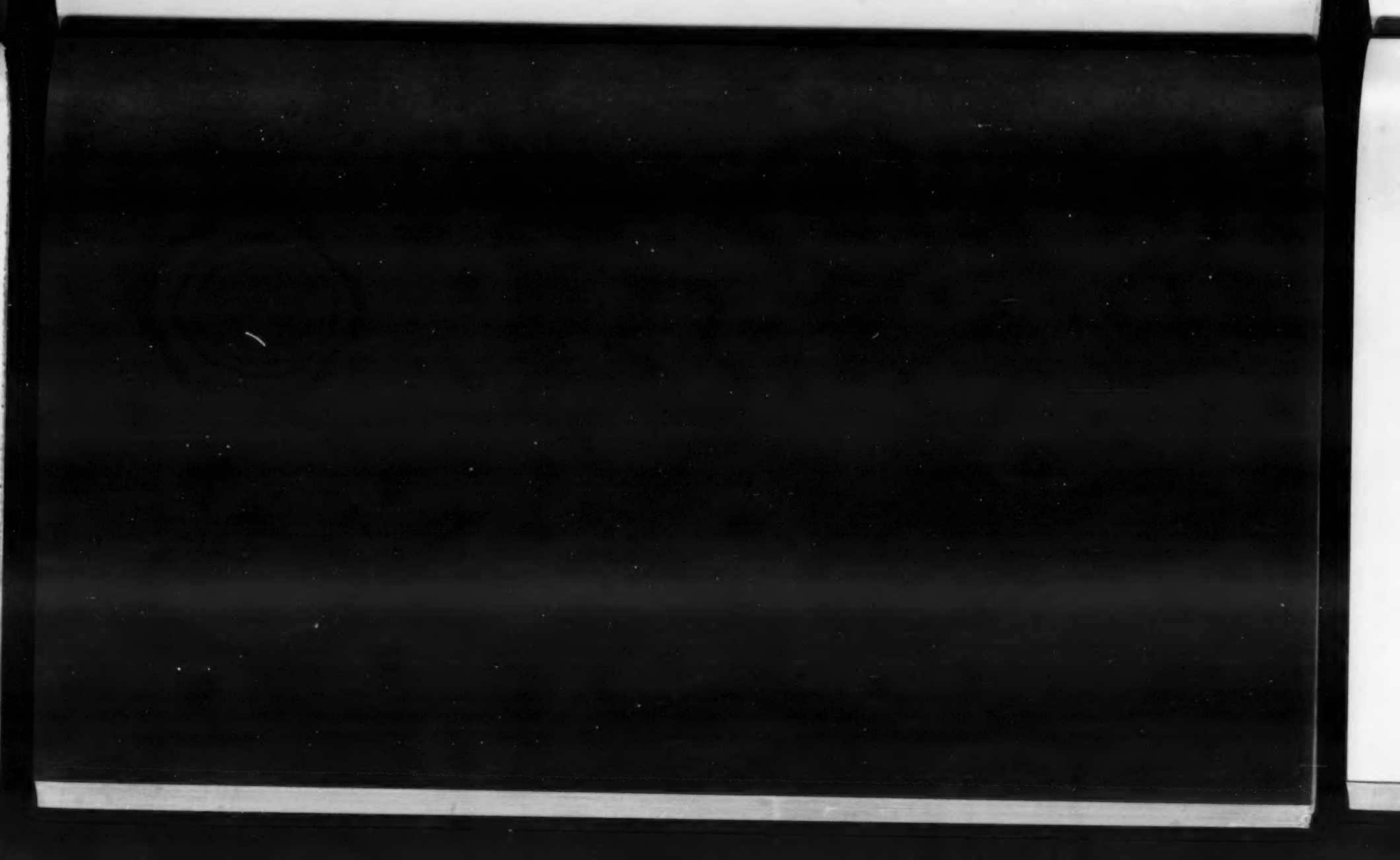

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BOECK'S DISEASE (BOECK'S SARCOID)

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BOECK'S SARCOID is a chronic disease, more common in Negroes than in white persons. It is characterized clinically by nodular lesions occurring in various parts of the body, notably in the lymph nodes, the lungs, the skin, the spleen, the liver and the bones. It has a protracted course, which is usually but not necessarily benign; sometimes it involves various parts of the body in progression, occasionally with remissions and exacerbations. It may be accompanied by fever, usually low grade, and characteristically by an increase of serum globulin, with a reversal of the albumin-globulin ratio. In fatal cases, tuberculosis, particularly of the lungs, is sometimes present. Pathologically, the lesion consists of disseminated small tubercle-like lesions composed almost entirely of histiocytes, the so-called epithelioid cells, among which are found giant cells resembling the Langhans type of giant cell. Caseation of any lesion is rare. Healing of the lesions occurs and is usually stated to be either by complete regression or by fibrosis and hyalinization.

The disease bears an unfortunate name, which had its origin in clinical assumption rather than in pathologic fact. The lesion does not have the features of a neoplasm and is now generally regarded as of inflammatory origin. It probably would be much better designated as Boeck's granuloma or Boeck's disease. Nevertheless, the term Boeck's sarcoid has served a useful purpose, calling attention to a definite disease entity, not to be confused with any other granulomatous lesions to which it might be compared.

Etiologically, this disease is obscure. The theory that it is a modified form of tuberculosis has many staunch adherents[1] and equally determined

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1. (*a*) Schaumann, J.: Brit. J. Dermat. **48**:399, 1936. (*b*) Bernstein, S. S., and Oppenheimer, B. S.: J. Mt. Sinai Hosp. **9**:329, 1942. (*c*) Longcope, W. T., and Pierson, J. W.: Bull. Johns Hopkins Hosp. **60**:223, 1937.

opponents.[2] The proponents argue that it resembles tuberculosis clinically, and from the standpoint of pathology they point to the frequency wtih which tuberculosis is proved to be present in the patients, and answer the objections based on the differences between the two diseases by asserting that Boeck's disease is a modified form of the classic tuberculosis, the modification being due to immunologic factors in the patient. The antagonists are not convinced that the two diseases have a common cause and refer to the almost uniformly negative results of tuberculin tests in cases of Boeck's disease, the failure to reproduce the disease in animals, the characteristic reversal of the albumin-globulin ratio and the peculiar involvement of the skin, the bones and the eyes without caseation. The objections also include the fact that the two diseases may coexist and that either one may precede the other.

There is a third group of writers exemplified by Ross,[2b] Scott and Robb-Smith[2c] and Scott[3] who, while admitting their lack of knowledge of any specific etiologic factor, regard the disease as a disturbance of the reticuloendothelial system, similar in some respects to the leukoses and to Hodgkin's disease. With these diseases, it is suggested, Boeck's disease has in common the proliferation of cells limited to those of mesenchymal origin, the involvement of spleen and lymph nodes and the absence of a known causative agent. In a morphologic consideration this view has definite merit, and while it does not offer any specific cause for the disease, nevertheless it classifies it in an understandable pathologic group, to which it also may have an etiologic relationship.

In all it seems best that, until the pathogenesis is determined beyond controversy, the causation be considered as unknown.

The pathologist finds this disease more frequently in biopsies than in necropsies. The number of necropsies described in the literature is not large. Consequently, the presentation of the postmortem observations made in 4 cases may be of some interest.

REPORT OF CASES

CASE 1.—A 61 year old white woman, known to have hypertensive cardiovascular disease, was admitted to the Goldwater Memorial Hospital because of a second episode of cerebral thrombosis. She died five days after admission. Physical examination gave essentially negative results except for her neurologic status. Autopsy revealed old and recent bilateral encephalomalacic areas. The liver was fatty. The kidneys showed moderate arteriolar sclerosis.

Of primary interest was a small scarred area at the inferolateral portion of the upper lobe of the right lung. Microscopically, this proved to consist of a hyalin-

2. (*a*) Kissmeyer, A.: Bull. Soc. franç. de dermat. et syph. **41**:1327, 1934. (*b*) Ross, J. M.: J. Path. & Bact. **37**:311, 1933. (*c*) Scott, R. B., and Robb-Smith, A.H.T.: St. Barth. Hosp. Rep. **69**:143, 1936.

3. Scott, R. B.: Brit. M. J. **2**:777, 1938.

ized fibrotic scar with moderate lymphocytic infiltration and anthracotic pigment. At the deeper edge of the scarred area were small localized collections of relatively large cells with eosinophilic cytoplasm and oval or rounded, relatively pale, vesicular nuclei. Many of these cells had coalesced to form multinucleated giant cells, some of which appeared to be of the foreign body type, while others were of the Langhans type. No necrosis was present.

The lymph nodes showed no gross abnormalities. Microscopically, a paratracheal node revealed a central fibrotic mass of dense hyalinized collagenous fibers containing considerable anthracotic pigment and some cleftlike spaces suggesting cholesterol. The remainder of the lymph node was infiltrated by clumps of epithelioid cells similar to those in the lung. Giant cells were much less common. No necrosis was present.

The spleen was natural. Granulomas were not found in other organs. Ziehl-Neelsen stains revealed no tubercle bacilli in lymph nodes or lungs.

Of special interest pathologically in case 1 was the finding in the lung of tubercles formed by large histiocytes, characteristic of Boeck's disease, conjoined to an old, hyalinized scar which had the appearance of a healed focus of tuberculosis. The Boeck's lesion, too, was of some duration and, although still in the cellular stage, showed early healing. This was manifested by the condensation of the cytoplasm of the individual cells and by the hyalinization of parts of the tubercles. This mode of healing by hyalinization within the tubercle is seen often in Boeck's disease. It was also of interest in this case that the disease was limited principally to the chest and that the spleen was uninvolved.

Case 2.—The patient, a Negro, was 41 at the time of death. Eight years previously he noted an eruption of the skin involving the mouth, the neck, the perineum and the thorax. The eruption was accompanied by generalized enlargement of the lymph nodes, especially of the femoral and inguinal lymph nodes.

Six years before death the patient was admitted to the New York Post-Graduate Medical School and Hospital primarily for antisyphilitic treatment. The result of a tuberculin test (dilution, 1:10,000) was reported as negative. A roentgenogram of the right ring finger showed a cystic degenerative change in a phalanx. On biopsy a lesion of the skin of the neck was diagnosed as Boeck's sarcoid, and the patient was presented at a meeting of the New York Academy of Medicine.[4]

In the next three years the patient was treated intermittently with roentgen radiation, and there was some decrease in the grandular enlargement. Three years before death he was admitted to Bellevue Hospital. A fine nodular infiltration of both lung fields was noted on roentgen examination, together with rarefaction of the bones of both hands and of the ribs. A tuberculin test showed a weak reaction, the patient responding to 0.005 mg. of purified protein derivative of tuberculin U.S.P. after thirty-six hours. The serum protein determinations were reported on two occasions to have shown: (1) albumin, 3.78 Gm., and globulin, 5.32 Gm. per hundred cubic centimeters; (2) albumin, 3.69 Gm., and globulin, 4.63 Gm.

Biopsy of a lymph node revealed many epithelioid tubercles (figs. 1 and 2). Surrounding and to a slight extent infiltrating the tubercles were marked deposits of an amorphous eosinophilic material resembling hyalinized collagen. Fibro-

4. Niles, H. D.: Arch. Dermat. & Syph. 40:1050, 1939.

blasts were not evident, and the appearance suggested that the collagen-like material was deposited by the epithelioid cells. There was no necrosis. The diagnosis was Boeck's disease.

One year before death the patient was readmitted to Bellevue Hospital because of the onset of signs and symptoms which subsequently proved to be due to subacute bacterial endocarditis. In the hospital the lesions of the skin were noted to have persisted, as had the enlargement of lymph nodes. The patient was transferred to Goldwater Memorial Hospital, where the findings were essentially similar. The result of a tuberculin test (dilution, 1:10,000) was negative. Biopsy of

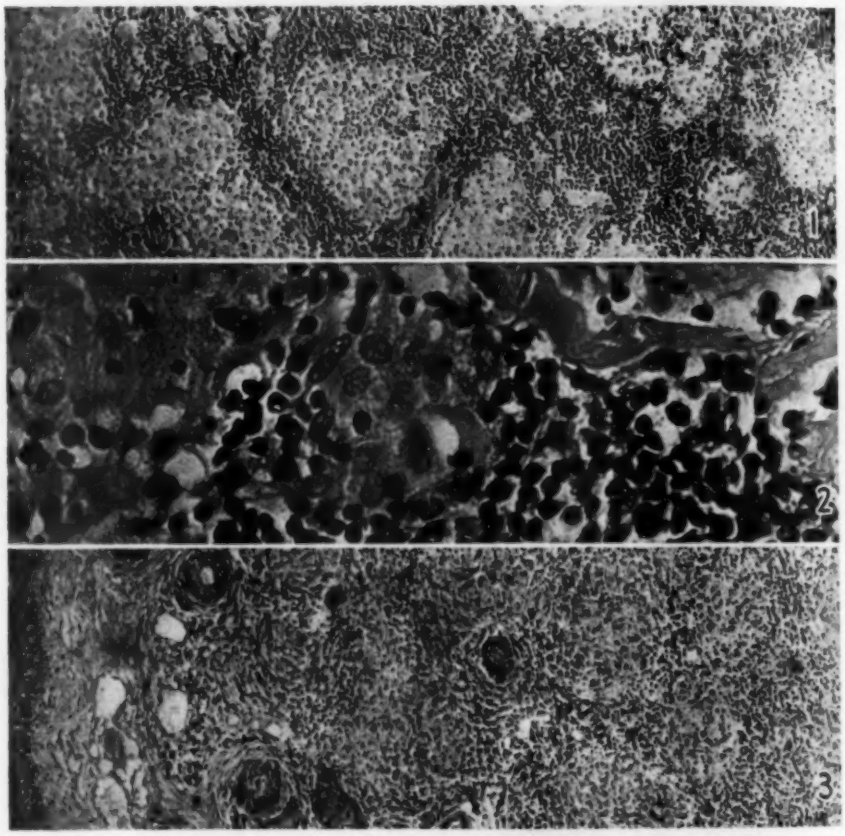

Fig. 1 (case 2).—Lymph node. The node was taken for biopsy in 1941, three years before death. It shows characteristic Boeck tubercles. In some areas the cytoplasm of the epithelioid cells is beginning to assume a homogeneous waxy appearance. Hematoxylin and eosin stain; × 100.

Fig. 2 (case 2).—Lymph node. The section is from the same biopsy specimen as that shown in figure 1, taken three years before death. Note the amorphous eosinophilic hyaline bands deposited within and between the tubercles. That the hyaline material is largely condensed at the periphery of the tubercle is clearly evident. Hematoxylin and eosin stain; × 550.

Fig. 3 (case 3).—Skin. The specimen was taken for biopsy eight months before death. Histiocytes are infiltrating the corium, forming tubercles. Hematoxylin and eosin stain; × 100.

a lymph node twelve months before death revealed changes considered characteristic of Boeck's disease. Eight months before death a biopsy of skin revealed, deep in the corium, conglomerate masses of epithelioid cells with a few giant cells. There was no necrosis.

The patient died as a result of his subacute bacterial endocarditis, which was found at autopsy to have involved the tricuspid valve. Mycotic aneurysms were noted in many branches of the pulmonary artery. Large hilar lymph nodes were seen. The spleen was enlarged, weighing 290 Gm.

Sections of the lymph nodes removed at autopsy revealed some to be almost identical with the biopsy specimen examined twelve months before death. Others revealed marked scarring with acellularity and calcification suggestive of a healed tuberculous lesion. Many others revealed no involvement of Boeck's disease, but showed edema, dilatation of the sinuses and hyperplasia of the reticuloendothelial cells. Sections of the spleen showed no definite lesions of Bock's disease. Sections of both apexes of the lungs revealed fibrotic scars and sections of the lower lobe of the right lung, some lymphocytic infiltration and small tubercles of mononuclear epithelioid cells characteristic of Boeck's disease.

Case 2 illustrates the migratory type of involvement of various organs in this disease in which new crops of tubercles may appear in some regions while those that occurred in others are regressing. In this case, as in the previous one, the disease was not the major cause of death; death was due to bacterial endocarditis. The ulcerous involvement of the right side of the heart is of interest, and one can speculate that the fibrosis of the lungs and the consequent strain on the right side of the heart were contributory to the localization of the bacterial process.

Case 3.—A Negro woman, 45 years old at the time of death, was known to have had hypertensive cardiovascular disease with mild congestive symptoms for eight years prior to death and to have suffered a cerebral accident with permanent left hemiplegia seven years prior to death.

Fifteen months before death a maculopapular eruption appeared over the face. It cleared somewhat with nonspecific therapy but never completely. At about the same time bouts of low grade fever began to occur, associated with malaise and vague pains of muscles and joints. Shortly thereafter, small lymph nodes, discrete, firm and easily movable, were noted in the cervical and inguinal regions. The spleen became palpable eight months prior to death. Roentgenograms of the chest revealed mottled infiltration of both lung fields and enlargement of the hilar glands. Roentgenograms of the hands disclosed osteoporotic changes but nothing specifically suggesting Boeck's disease. The result of a Mantoux test made with old tuberculin (dilution, 1:1,000) was negative. Chemical examination of the blood revealed 1.3 Gm. of albumin and 4.5 Gm. of globulin per hundred cubic centimeters of serum.

Eight months before death biopsy of skin showed nodules of epithelioid cells in the corium (fig 3). There were no giant cells and no necrosis.

Death was due to pneumonia and pneumococcic meningitis.

Autopsy revealed, in addition to the diffuse pulmonary infiltration, a number of small, firm, gray-white nodules, about 2 mm. in diameter, scattered throughout both lungs and concentrated in the lower lobe of the right lung. The spleen was enlarged, weighing 350 Gm. The cervical, mediastinal and periaortic lymph nodes and lymph nodes in the hilar region of the liver were enlarged and discrete.

Sections of the lung, most characteristically those of the lower lobe of the right lung, revealed conglomerate masses of an amorphous eosinophilic material resembling hyalinized collagenous fibers. In favorable locations, small individual clumps of material were easily discernible within the larger conglomerations. These clumps were similar to those noted in the biopsy eight months before death. In other areas a more diffuse and irregular type of fibrosis was seen. Interspersed between the clumps of eosinophilic material were considerable numbers of large monocytes, lymphocytes and a few plasma cells. Many multinucleated giant cells

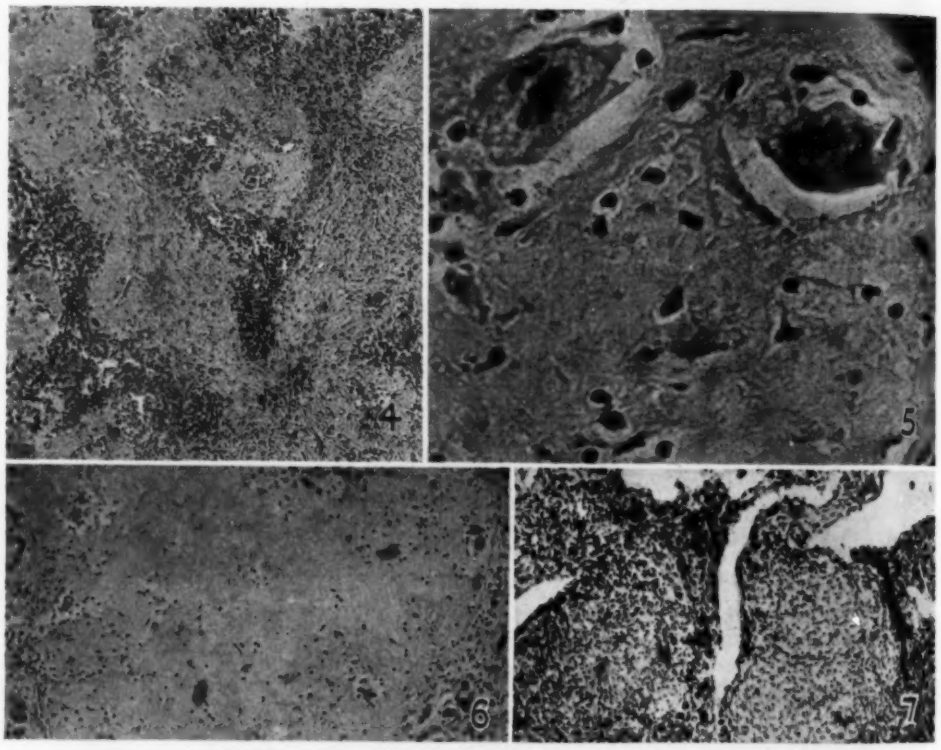

Fig. 4 (case 3).—Spleen at necropsy. Many large deposits of hyaline material may be noted. Multinucleated giant cells are present. Hematoxylin and eosin stain; × 50.

Fig. 5 (case 3).—Spleen under higher magnification. Hyaline bands and giant cells are seen. Hematoxylin and eosin stain; × 550.

Fig. 6 (case 3).—Liver. It shows a hyalinized lesion of the portal area, illustrating the healed stage of Boeck's disease. Hematoxylin and eosin stain; × 100.

Fig. 7 (case 4).—Lung. A number of active Boeck tubercles are noted in the septums, which are generally thickened. Hematoxylin and eosin stain; × 100.

were noted, some of which were of the Langhans type. The bronchi showed marked chronic inflammatory changes. The walls of the arteries showed moderate sclerotic changes.

The lymph nodes revealed on section, deposited throughout, small clumps of amorphous eosinophilic material similar to that in the lung. In areas these clumps

formed conglomerate masses. The eosinophilic material stained blue with azocarmine, as it did in all other locations. A few multinucleated giant cells were present.

The spleen presented an essentially similar picture (figs. 4 and 5). Masses of eosinophilic material were noted in great numbers, to a large extent replacing the normal splenic structure. A special feature of the involvement of the spleen was the presence of many multinucleated giant cells, mainly of the foreign body type, in the midst of the hyalinized areas.

Sections of the liver revealed slight fatty infiltration, slight diffuse fibrosis and moderate lymphocytic infiltration within the portal canal. Clumps of an amorphous eosinophilic material were seen deposited within some of the portal canals (fig. 6). Stains for amyloid and acid-fast bacilli revealed none.

For a long time this patient had suffered from hypertensive cardiovascular disease with multiple cerebrovascular accidents. At autopsy she showed well defined lesions of Boeck's disease as well. Although the clinical symptoms referable to this disease had begun only fifteen months before death, nevertheless the lesions presented definite evidence of healing. The outstanding pathologic feature of this process was the well defined hyalinization of the lesions in the lungs, the liver, the spleen and the lymph nodes. The major cause of death was pneumococcic meningitis, to which Boeck's disease was not manifestly related as an etiologic factor.

CASE 4.—The patient was a Negro man, 20 years old at the time of death. Eleven years previously a roentgenogram of the chest was taken because tuberculosis had developed in his sister. It was reported as showing no evidence of tuberculosis. Ten years before death a second roentgenogram of the chest, taken because of cough, dyspnea, loss of appetite and loss of weight, revealed enlargement of the hilar nodes and bilateral peribronchial infiltration in the middle third of the left lung and in the lower part of the right lung. Physical examination revealed generalized lymphadenopathy, particularly of the cervical nodes. There were crepitant rales in the lower lobe of the right lung and over the apex of the left lung. The patient was admitted to Bergen Pines Hospital. Sputum and aspirated gastric content were free from tubercule bacilli when examined on repeated occasions. Two tuberculin tests were reported to have given negative results, although subsequently a positive tuberculin reaction was described. Nine years before death a biopsy of a lymph node was reported as showing tuberculous adenitis. These slides were reviewed in our laboratory and showed the tissue to contain only epithelioid nodules, which we considered characteristic of Boeck's disease.

Later that year an axillary lymph node broke down, yielding a caseous material which showed tubercle bacilli on culture. The patient remained in the hospital for two more years and then left against advice. At discharge a roentgenogram of the chest showed only a slight decrease of the size of the mediastinal shadow and of the peribronchial infiltration.

The patient was apparently well for the next seven years, working as a messenger boy. Two years before death he began to notice progressive dyspnea on exertion.

On admission to the Goldwater Memorial Hospital he was found malnourished and underdeveloped. The veins of the neck were distended. The chest was emphysematous. The liver was enlarged. There was no peripheral edema. The fingers were not clubbed. The admission diagnosis was emphysema and cor pul-

monale (dilatation and hypertrophy of the heart due to pulmonary disease). Significant laboratory data included: serum albumin, 2.5 Gm. per hundred cubic centimeters; serum globulin, 3.3 Gm. Examination of the sputum, including guinea pig inoculation, revealed no evidence of the presence of tubercle bacilli. Death resulted from cardiac failure.

At autopsy the lungs were emphysematous. Moderate tubular bronchiectasis was present in all lobes. The heart was enlarged, especially on the right side. The spleen was enlarged, weighing 330 Gm., and was firm.

Microscopically, the lungs revealed marked emphysema, bronchiectasis, areas of atelectasis and interstitial fibrosis (fig. 7). Numerous epithelioid nodules were found, many of which showed fibrosis and hyalinization involving usually a part of the tubercle, especially the peripheral portion. The liver contained numerous tubercle-like lesions of similar appearance, and hyaline deposits were noted. The spleen contained similar small tubercles. The kidney did not show any frank tubercles, but a rare nodule containing hyaline bands surrounded by lymphocytes was noted in the cortex. The lymph node contained similar epithelioid tubercles, which showed an occasional giant cell. No caseation was seen, nor were large scarred or calcified areas observed as in frank tuberculosis. Guinea pig inoculation of material taken from the lungs and lymph nodes produced no evidence of the presence of turbercle bacilli.

The most significant question in case 4 is whether the major lesions were tuberculous. In support of the assumption that they were might be the fact that the patient had a caseous axillary node from which acid-fast bacilli were isolated. However, the other facts in the case are distinctly against this assumption:

1. The disease manifested itself as an extensive involvement of the lung parenchyma. At the age of 10 a tuberculous lesion of this magnitude would indicate severe disease. Yet the result of a tuberculin test made at the time of admission was negative as were the results of repeated examinations of sputum and gastric washings. The rapid improvement observed clinically is also unusual for this type of tuberculosis at this age.

2. Besides the numerous Boeck tubercles diffusely infiltrating the lung, the other outstanding feature was the marked pulmonary emphysema. The mechanism of this emphysema must be attributed to the aforesaid diffuse infiltration of the lung and to the associated septal fibrosis. Pulmonary emphysema of this extent is certainly unusual for classic tuberculosis and its presence as well as that of cor pulmonale, without large areas of pulmonary fibrosis, is much more to be expected in Boeck's disease than in classic tuberculosis.

3. The evidences of healing seen in this case, including the hyalinization of tubercles and the deposition of hyaline bands, are similar to the findings in our other 3 cases.

4. The origin of the tuberculous infection of the axillary lymph node would most probably have been a hematogenous spread from the chest. If the lungs were diffusely tuberculous, they probably would have been the primary site, and several years later should have shown evidence of

diffuse fibrosis or extensive ulceration. And yet, nine years after the removal of the caseous node the lungs were found to be in an unhealed cellular stage without showing the usual changes of healed tuberculosis or of caseation. One can only conclude that the pulmonary lesion found at autopsy was a type by itself and that the bacilli isolated from the axillary node probably had their origin in a small unrelated pulmonary lesion or in some other visceral focus.

In all, therefore, this case has clinically several features that cannot be entirely explained by a tuberculous causation. Morphologically, the divergence between the two disease states is even greater, and the pathologic diagnosis at the time of death was clearly Boeck's disease.

COMMENT

The 4 cases described were all diagnosed at autopsy as instances of Boeck's disease. In cases 2 and 3 the diagnosis was made clinically. In case 1 there were insufficient clinical data to warrant a diagnosis of Boeck's disease, as the patient was admitted in coma and lived for only a short time after admission. Regarding case 4, we feel that the diagnosis could have been made ante mortem on the basis of biopsy of a lymph node. In only 1 case was Boeck's disease the major cause of death (case 4) and even then only indirectly, through corpulmonale due to pulmonary Boeck's disease. Tuberculosis was neither a major nor a contributory cause of death in any of the cases.

This series is too small for any conclusive analysis. Nevertheless, certain impressions are of interest. Of the 4 patients, 2 were males and 2 were females. Three of the patients were Negroes. The high proportion of Negro patients is in agreement with the well known frequency of this disease in the Negro race. The ages were 61, 43, 40 and 20 respectively. The organ involvement was as follows: lungs, 4 cases; lymph nodes, 4 cases; liver, 2 cases; spleen, 2 cases; skin, 2 cases; kidney, 1 case.

Although all 4 patients showed pulmonary involvement, nevertheless the amount of involvement of the lungs varied greatly, from focal lesions in 3 cases to extensive and diffuse infiltration in 1 case (4). Similarly, the involvement of lymph nodes varied. It was not uncommon to find some lymph nodes involved and others completely free from disease. A healed tuberculous lesion was found in 2 cases. There was also a history of tuberculosis in another case (4). Caseation was not found in any of the cases. Calcification was seen in 1 case but was attributed to a previous tuberculous lesion which had healed.

The eyes and the parotid glands were not examined pathologically. No definite lesions of bones were encountered, but, because of autopsy limitations, most of the suspected bones were not examined. Characteristic bone changes, however, were seen on roentgenologic examination in

2 cases (2 and 4). Evidence of healing of Boeck's lesions was seen in all cases. The healed lesions resembled one another in whatever organ they were found.

HEALING OF BOECK'S DISEASE

Since in these 4 cases the lesions had existed for some time, we became interested in ascertaining the degree of healing in them. A review of the literature reveals that little has been written about the terminal or healed stage of Boeck's disease as compared with the accounts of the acute or active stage. The question naturally arises as to what becomes of the lesions in the later stages of the disease. Do they disappear altogether, so that the involved tissues are restored to their original state, or do they leave scars or traces when they heal, and, if so, can the residua be differentiated from the end results of other granulomatous processes?

Longcope and Pierson[1c] stated that Boeck's lesions retain the appearance of epithelioid tubercles in skin and lymph nodes that have been affected for months or even years. They stated, however, that healing may occur through fibrosis.

Hollister and Harrell[5] described the lesions in their case as being practically wholly made up of eosinophilic material, which was identified as fibrous tissue.

Reisner[6] found that many of the lesions in the various organs had regressed to the point of complete disappearance of all objective manifestations in 9 of 28 cases. His statement was based, however, on clinical and roentgenologic findings alone.

Klemperer, in a discussion of a case described by Bernstein and Oppenheimer,[1b] noted that in that case the postmortem examination did not show any evidence of a lesion that resembled the epithelioid tubercle found in sarcoidosis. Instead, the conspicuous feature of the case was "the peculiar hyaline bands found in lymph nodes, spleen and lungs." The study of several cases of undoubted sarcoidosis had convinced him that the end stage of this morbid process was characterized by hyalinization.

Pinner,[6a] in describing the healing phase of Boeck's disease, directed attention to fibrotic and hyalinized lesions.

Tice and Sweeney,[7] commenting on their case, stated that from a roentgenologic standpiont, as the lesions become chronic, their soft nature disappears and a stringy appearance not unlike fibrosis develops instead. They further stated that pathologic examination of the lymph nodes showed broad bands of fibrous tissue with varied amounts of lymphoid

5. Hollister, W. F., and Harrell, G. T.: Arch. Path. **31**:178, 1941.

6. Reisner, D.: Am. Rev. Tuberc. **49**:289 and 437, 1944.

6a. Pinner, M.: Pulmonary, Tuberculosis in the Adult, Springfield, Ill., Charles C Thomas, Publisher, 1945, p. 342.

7. Tice, F., and Sweany, H. C.: Ann. Int. Med. **15**:597, 1941.

tissue and a few giant cells in different stages of evolution. In the lungs and beneath the pleura there were small plaques of fibrous tissue. There were zones of fibrous tissue containing giant cells.

Rubin and Pinner,[8] in a review of the literature of sarcoidosis, described a case of Mylius and Schurmann. There was a mediastinal tumor mass in which there were foci of epithelioid cells surrounded by massive connective tissue, part of which was hyalinized.

Schauman[1a] stated that in the evolution of the lesion the granulomatous tissue is transformed into a fibrous tissue, with consequent partial or complete disruption of the organ.

The findings in our cases relative to the healing of lesions by fibrosis and hyalinization corroborate the general descriptions of these writers and particularly those of Klemperer and Pinner. We do not believe that this is the only mode of healing. Complete regression with disappearance of all traces of the disease in all probability occurs. In a few lymph nodes we found faint areas of hyalinization suggesting the possibility that Boeck's disease had involved these structures but that most of the pathologic changes had disappeared, leaving only small tokens of a more extensive disease. It can be understood that if a tissue is restored to complete normality, it would be difficult to prove antecedent involvement unless one had periodic biopsy specimens from the same tissue or organ. We had none such available.

Various stages of healing were observed in these 4 cases. We wish to describe the findings in some detail.

Lymph Nodes.—The fully developed Boeck tubercle consists of a mass of reticulum cells, among which may be found one or more giant cells. The mass is embedded in lymphoid tissue, so that a ring of remaining lymphocytes may surround the collection of reticulum cells. The latter are fairly large, with a grayish pink cytoplasm (hematoxylin and eosin stain) and a vesicular nucleus. The cells are elongated or polyhedral. Generally, they do not present definite cell outlines, and on hematoxylin-eosin staining the nuclei appear to be embedded in a syncytial type of cytoplasm. Fibrillar processes are seen to extend from the cell bodies. Reticulum stains show reticulum fibers to be present between the cells.

The beginning of healing appears to be an increase in the eosinophilic staining properties of the reticulum cells (fig. 3). Areas in the tubercle assume a more prominent reddish tint. With further healing the cytoplasm of the cells becomes more eosinophilic and homogeneous. The nuclei decrease in number, and those that remain are smaller, darker and more elongated. In this state the tubercles are still cellular. With the azocarmine stain, coarse blue fibers are now found in the tubercle. The

8. Rubin, E. H., and Pinner, M.: Am. Rev. Tuberc. 49:146, 1944.

tubercle then becomes denser, more hyaline and less cellular. Masses of hyaline material or hyaline bands appear in the remaining lymphoid tissue and especially at the periphery of the tubercle (fig. 4). Some of these bands merge with the hyaline deposits of the tubercle itself, replacing cellular cytoplasm and nuclei. During this process the giant cells fare better than their fellows. They are frequently seen as the remaining outposts in an area of hyaline devastation. Eventually they, too, disappear in the engulfing hyalinization, so that hyaline masses alone are left. These may still show in areas vague outlines of tubercles. Occasionally the hyaline masses are not entirely acellular but consist of interlacing hyaline bands separated by rows of compressed lymphocytes or histiocytes. Finally the lesion becomes converted into an acellular hyaline scar (fig. 6).

Lungs.—The pulmonary process in general is similar to that described in the lymph nodes. Fibrosis, as well as hyalinization, is noted here. Some of the alveolar septums may become fibrosed. In addition, there may be seen fibrous scars, which are unusually small and frequently hyalinized. They may be found especially in the hilar region of the lung parenchyma and in the pleura. Occasionally, hyalinized tubercles which have become conglomerate retain vague outlines. In spite of the extent of the lesion, calcification was not seen in typical lesions of Boeck's disease.

Spleen.—The lesion of the spleen resembles that of the lymph node. The enlargement of the organ and its increased density may suggest the presence of amyloid. Special stains, however, will fail to corroborate such an impression. In fact, the hyalinized appearance of the spleen, when present, may be typical of this disease. It must be pointed out, however, that the spleen need not be involved. In only 2 of 4 cases was this organ invaded by either tubercles or hyaline deposits.

Other Organs.—In 2 of our cases (2 and 4) we found eosinophilic, waxy, apparently healed nodules in the liver which were similar to those seen in the spleen. They seemed to be most prominent in the portal regions. Nevertheless, they were also found in the parenchyma proper.

In the 4 cases, definite lesions were not found anywhere else in the body. One suggestive lesion was found in the kidney. In 1 case (3) an insufficient amount of bone removed at autopsy showed no evidence of Boeck's disease.

We can therefore say that as far as healing of Boeck's disease is concerned, fibrosis and particularly hyalinization are seen as end stages. The hyalinization appears to be an integral part of the process of healing within the epithelioid tubercle. It is apparently not related to necrosis as in the tubercles of classic tuberculosis but is concerned with replacement of existing cellular cytoplasm. We do not believe that this is the only mode of healing but we feel that it is a characteristic one.

SUMMARY AND CONCLUSION

Four cases in which autopsy showed lesions of Boeck's disease are presented, and the pathologic observations are discussed.

In spite of the varied clinical course, the lesions resembled one another in many respects in the acute as well as in the healing stages.

Healing of these lesions may occur in several ways, including complete regression. However, fibrosis and hyalinization without calcification were seen in all the cases presented. It is therefore postulated that this is a common form of healing in cases of this disease. The healing lesions themselves, particularly in the spleen and the lymph nodes, may be sufficiently characteristic to suggest the diagnosis of Boeck's disease, which should be corroborated by the finding of more acute lesions in other organs or tissues and by the clinical facts.

Although at many points this disease resembles tuberculosis, nevertheless its causation and pathogenesis have not been definitely established.

Morphologically, the lesions of Boeck's disease can be differentiated from other granulomas, including those of classic tuberculosis, both in the acute and, in many cases, in the chronic or healing stages. In the acute stage the predominance of histiocytes occurring in discrete tubercles without any marked accompaniment of lymphocytes and without necrosis is characteristic. In the healing and healed stages the fibrosis and the marked tendency toward hyalinization within the tubercles, without calcification, and the retention of occasional outlines of tubercles in hyalinized areas are suggestive of Boeck's disease.

CALCIFIC DISEASE OF THE AORTIC VALVE

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INCREASING INTEREST is being directed toward diseases of the aged as the population becomes progressively older. Calcific aortic stenosis forms an appreciable percentage of valvular cardiac disease in the older age group. It is found in about 1 per cent of routine autopsies,[1] and it is three times as frequent after the age of 50 as before.[2] Increasing interest in this disease is evidenced by numerous papers on the subject in the past two decades and one recent monograph.[2] Most reports deal with clinical aspects of the process. Anatomic studies have yielded somewhat divergent results regarding etiologic factors.

Monckeberg,[3] in 1904, gave a careful description of the gross and microscopic features of the process in 32 cases of the disease, in which varying degrees of valvular involvement were present, and concluded that the changes were essentially degenerative in origin. Subsequent workers,[4] largely on the basis of histologic evidences of previous inflammatory disease found after careful study of numerous areas of the heart, concluded that the process was essentially of rheumatic origin. This was the conclusion reached by Karsner and Koletsky[2] in their study of 200 cases. Some investigators,[5] using similar methods, found in several instances no stigmas of previous inflammatory disease and concluded that in these cases the lesion represents a purely degenerative process.

Most of the studies, however, have been done on full-blown cases of the disease with severe stenotic lesions at the aortic orifice. Relatively little work has been directed toward examining calcifications of the aortic valves of unselected persons without cardiac disease. Ashworth[6] reviewed 500 autopsies and studied 100 hearts grossly, finding valvular

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1. Dry, T. J., and Willius, F. A.: Am. Heart J. **17**:138, 1939.
2. Karsner, H. T., and Koletsky, S.: Calcific Disease of the Aortic Valve, Philadelphia, J. B. Lippincott Company, 1947.
3. Monckeberg, J. G..: Virchows Arch. f. path. Anat. **176**:472, 1904.
4. Clawson, B. J., and Bell, E. T.: Am. J. Path. **2**:193, 1926. Clawson, B. J.: Arch. Path. **12**:889, 1931. Clawson, B. J.; Noble, J. F., and Lufkin, N. H.: Am. Heart J. **15**:58, 1938. Hall, E. M., and Ichioka, T.: Am. J. Path. **16**:761, 1940.
5. Sohval, A. R., and Gross, L.: Arch. Path. **22**:477, 1936.
6. Ashworth, C. T.: Arch. Path. **42**:285, 1946.

sclerosis in 63 per cent of these hearts, 7.2 per cent of which had severe degrees of the process. He found the process more common and extensive in the older age groups. Epstein[7] reviewed 125 cases of nonrheumatic heart disease and found calcifications of the aortic valve present in 27 cases (21.6 per cent). All the patients were over 50 years. Giese[8] carefully described the gross and microscopic features of calcification occurring in the mitral ring and the aortic valve in 7.5 per cent of 700 autopsies.

It is of importance to study the subclinical or intermediate stages of the process in unselected adults, for if the changes are purely degenerative one would expect to find a gradual increase of incidence and severity with advancing age paralleling that observed in other degenerative vascular changes elsewhere in the body. One could in addition study the histologic features of early or minimal lesions and obtain evidence regarding the genesis of the process. It was with these points in mind that this study was undertaken.

MATERIAL AND METHOD

The material consisted of 100 hearts from unselected, consecutive autopsies made on adults in the department of pathology of the Stanford University School of Medicine. The average age of the patients in the series was 58 years; the youngest was 26 years and the oldest 86 years of age; 33 per cent of the total number were females. The aortic valve and mitral ring were dissected free from each heart, spread out with pins and preserved in a 4 per cent solution of formaldehyde. They were then studied carefully for sclerotic changes and particularly for deposits of calcium. The results were plotted diagrammatically on a separate sketch sheet for each heart. To insure greater accuracy in locating tiny deposits of calcium small roentgenograms were taken of each specimen. This technic has been used previously.[9] Exposures were made for five seconds at 45 kilovolts and 15 milliamperes. In this way calcium particles as small as 2 mm. in diameter could be detected and accurately localized. In several instances calcium particles which were not evident on gross examination were demonstrated by this method. It was felt that a study of the presence, the size and the extent of calcifications visualized in this way would be a more objective method than attempting to estimate or "grade" the degree of sclerosis as done previously.[6] In addition, sections for histologic study were taken from each valve. Each heart was studied carefully for gross evidence of previous rheumatic disease. Multiple blocks of tissue were removed in each case according to the method described by Gross[10] and were studied when indicated. Finally the clinical records of the cases were examined for additional information.

7. Epstein, B. S.: Arch. Int. Med. **65**:279, 1940.
8. Giese, W.: Beitr. z. path. Anat. u. z. allg. Path. **89**:16, 1932.
9. Fertman, M. H., and Wolff, L.: Am. Heart J. **31**:580, 1946.
10. Gross, L.; Antopol, W., and Sacks, B.: Arch. Path. **10**:840, 1930.

ANATOMIC FINDINGS

Of the 100 hearts studied in this manner, 46 had demonstrable calcium deposits in the aortic valves. After careful study 11 of these hearts were eliminated because of gross lesions or unquestionable microscopic evidence of previous rheumatic disease. One heart had a bicuspid aortic valve with a severe degree of calcification. This left a total of 34 in which no indication of previous rheumatic heart disease could be found but which showed definite, grossly demonstrable calcium deposits in the aortic valves. The average age of the 34 hearts of this group was 67 years, which is definitely higher than the average age of the entire series (58 years).

An analysis of the age distribution of the hearts possessing such calcium deposits is indicated in figure 1. No deposits were found in any hearts under 40 years but from 60 years on most of the hearts contained calcium masses.

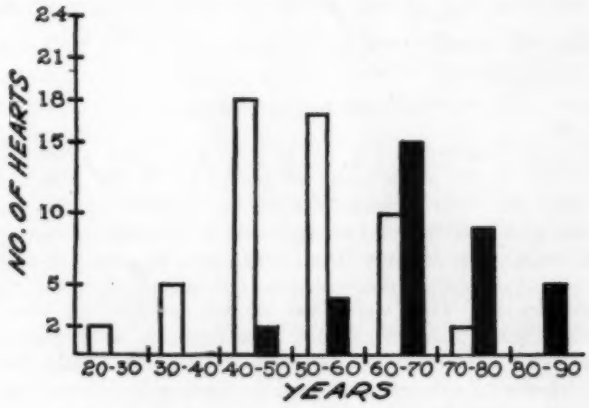

Fig. 1.—Age distribution of hearts studied for nonrheumatic calcification of the aortic valve. Blank columns represent hearts in which the aortic valve was free of calcification; black columns, hearts in which the aortic valve showed nonrheumatic calcification.

Not only does the frequency of calcium deposits increase with advancing years but the extent of the deposits increases as well. By examining the roentgenograms of the valves it was relatively simple to grade the deposits into two degrees—slight, or grade 1, and marked, or grade 2—as determined by the actual size and number of the calcium particles. The average age of the hearts showing grade 1 deposits was 61 years, while the average age of those showing grade 2 deposits was 74 years. Seventeen hearts were present in each group.

If the process of valvular calcification parallels degenerative changes elsewhere in the vascular system, some correlation between this process

and arteriosclerosis elsewhere might be expected. Although it is difficult to obtain an accurate objective measurement of the degree of arteriosclerosis in large blood vessels, a rough estimate of the degree of severity of the process may be obtained by measuring their content of calcium. In the accompanying table a rough correlation can be found between the presence of valvular calcifications and a high calcium content of the aorta. As might be expected, there is an increase of aortic calcium with advancing age. In addition the aortas from persons having calcifications in the aortic valves contain a greater amount of calcium than the aortas from persons with normal aortic valves. Also the aortic calcium content is higher in those in whom the degree of valvular calcification is marked as compared with those in whom the degree is slight. Approximately equal average age groups are compared to eliminate difference due to age alone.

Previous reports have indicated that calcific aortic stenosis is more common in males. Karsner and Koletsky[2] suggested that this merely re-

The Calcium Content of the Aorta and the Degree of Calcification Present in the Aortic Valve

	Hearts	Average Age	Calcium Content per Gram of Aorta, Mg.*
No calcium in aortic valve	10	44.0	19.7
No calcium in aortic valve	9	65.3	27.4
Calcium present in aortic valve	14	66.4	44.7
No calcium in aortic valve	6	63.6	26.5
Mild calcifications present	9	63.9	41.2
No calcium in aortic valve	7	71.7	25.9
Large calcifications present	5	71.0	48.1

*Calcium is reported as milligrams per gram of the descending portion of the aorta dried to constant weight after removal of the adventitia.

flects the greater percentage of males in the autopsy room population, and after correcting the ratios for his series of 200 cases, concluded that there is no significant preponderence of males over females in any of the decades of life. The results of the present study confirm that confusion. Thirty-three per cent of the entire 100 persons whose hearts were studied were females, and of the 34 with calcification of the aortic valve, 11, or 32.2 per cent, were females, indicating a fairly equal distribution between sexes. However, when the 34 were divided into two groups, those with prominent calcific deposits and those with minor degrees of calcification, it was found that 17.7 per cent of the former and 41.1 per cent of the latter group were females; the ratio of males to females was 3.52 to 1 and 1.43 to 1, respectively. These figures suggest that the process may be more severe in males, although the series is too small to allow any significant statistical analysis.

The gross appearance of the valvular changes is similar to that described by previous workers (Monckeberg,[3] Margolis and co-workers,[11] Ashworth[6] and others). Minor changes involved the aortic aspect of the valve at the angle of insertion in the base of the valve pocket. They consisted of the appearance of a pale yellow deposit extending the length

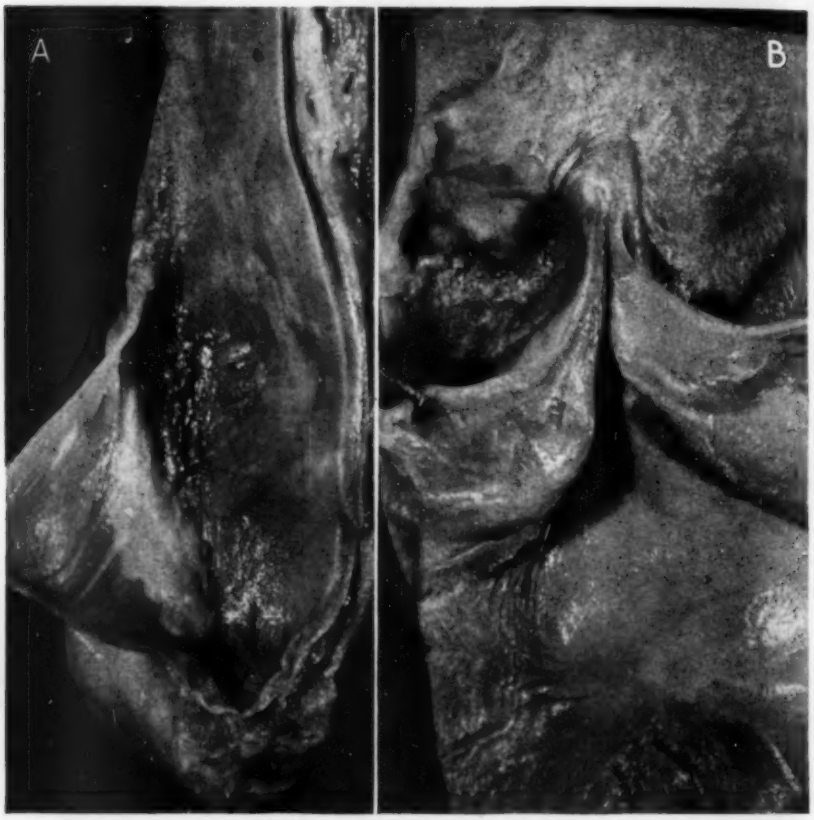

Fig. 2.—In *A* the aortic valve has been bisected longitudinally and a leaflet pulled to the left to reveal a deposit of lipid at the angle of insertion. The deposit is more marked near the sides of the pocket. The aorta lies to the right.

B, close-up of the bisected aortic valve, showing the prominent ridging, particularly at the sides of the pocket, formed by the collagenous alterations at the angle of insertion. The aorta is above; the ventricle, below.

of the valve pocket. Occasionally the deposit was more prominent toward the sides of the pocket. The borders of the deposit were smooth and distinct (fig 2*A*). At the same time there was present a distinct thickening, with increased rigidity, of the valve leaflet at its insertion point. This appeared to be dependent on a change in the collagenous

11. Margolis, H.; Ziellessen, F. O., and Barnes, A. R.: Am. Heart J. 6:349, 1930.

supporting tissue of that area rather than on the subintimal deposit of lipid. From the internal aspect of the sinus this alteration had the effect of filling in the acute angle at the insertion point. From the external and ventricular aspect a distinct rigidity could be felt easily by passing one's finger over the base of the valve from the ventricular endocardial surface toward the aorta. However, no involvement of the ventricular surface of the valve leaflet could be found. The intima remained smooth and glistening and free from deposits of lipid. This ridging often extended along the entire line of insertion of a leaflet and frequently was more prominent in the lateral portions of the leaflet rather than in the exact center (fig. 2*B*). Measurements of this palpable ridge revealed a height of from 1 to 4 mm., with an average of 2.5 mm. This could obviously produce a slight narrowing of the aortic orifice, but what role it plays in the production of murmurs can only be speculated on.

In more severe degrees of change the deposit of pale yellow lipid at the base of the valve leaflet is wider and extends up into the leaflet itself. This extension was usually accompanied by only a slight palpable thickening of the area. The ventricular aspect remained uninvolved. A lesser degree of extension of the deposit was noted in the posterior wall of the pocket, but it was almost never confluent with the atherosclerotic changes around the coronary ostiums at the base of the aorta.

Gross deposits of calcium were usually first visible in the thickening at the angle of insertion described (fig. 3*A*). They frequently appeared slightly to one side of the middle of the cusp where, as mentioned before, the preexisting alterations were frequently more marked. The smaller calcium particles were 2 to 3 mm. in diameter, smooth and beadlike and projected above the surface of the deposit at the base of the pocket. As they increased in size, they bulged into the valve pocket, extending along the line of insertion and extended up into the aortic aspect of the leaflet. They did not extend into the aortic wall of the sinus. Occasionally, calcium deposits appeared on the aortic aspect of the leaflet, unconnected with the calcification at the base. The advanced process fills the sinus with calcium masses projecting into the space from the now broadened line of insertion and the aortic aspect of the leaflet. The net result is a stiffened leaflet and an unyielding, stony-hard mass encroaching on the aortic orifice. It is possible that minor degrees of involvement may produce changes in heart sounds and murmurs without marked alterations in circulatory dynamics.

In agreement with previous studies, only minimal degrees of adhesion of valve cusps were found, and no rolling or thickening of the free edge of the leaflets was present even in many valves with more severe degrees of calcification.

The histologic appearance of the normal aortic valve has been well described by Gross and Kugel.[12] The microscopic appearance of markedly calcified valves is well described by Margolis,[11] Sohval and Gross[5] and others. However, the histologic appearance of the intermediate stages of the calcific process has received little attention. The most careful and complete descriptions of the changes are those by Monckeberg[3] who studied 32 cases of varying degrees of sclerotic change and by Giese,[8] who described the alterations occurring in the mitral ring as well. Ash-

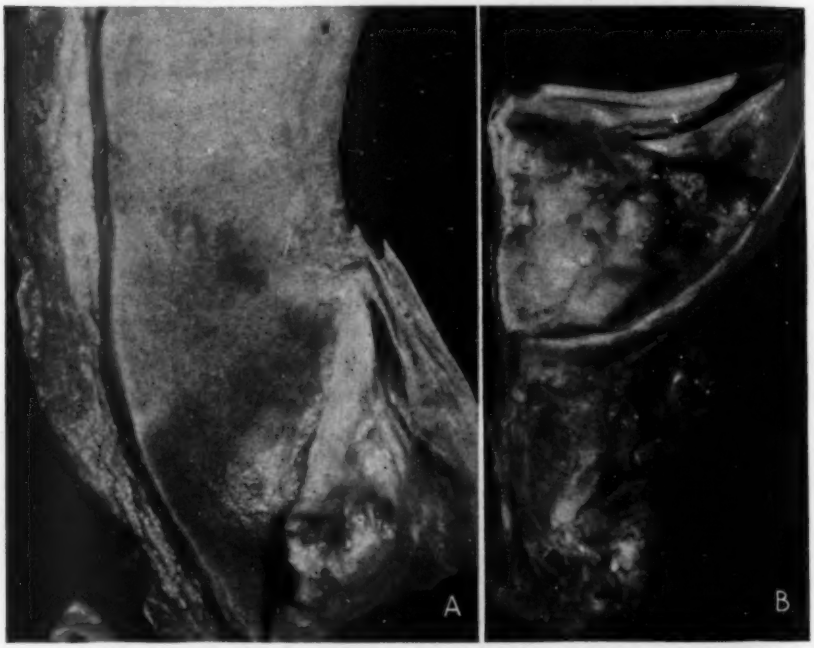

Fig. 3.—*A*, view similar to that in fig. 2*A*, showing, in addition, irregular calcium masses in the base of the pocket and extending into the aortic aspect of the leaflet.

B, view of a bisected aortic valve with a portion of the posterior wall of the sinus cut away to show calcium extending up into the valve leaflet.

worth[6] gave a brief description of the microscopic observations in his series of 100 cases. It was felt that a systematic histologic study of a large number of valves showing varying amounts of sclerosis would give some information as to the genesis of the lesion.

In the present series blocks for histologic study were cut from representative areas of the aortic valve in each case. Four per cent formaldehyde solution was used as a fixative. Valves showing gross calcium particles were decalcified for twenty-four hours in 2 per cent nitric acid and 4 per cent formaldehyde solu-

12. Gross, L., and Kugel, M. A.: Am. J. Path. 7:445, 1931.

tion. The specimens were embedded and cut so as to retain as much as possible the normal anatomic relationships. Each section was stained with hematoxylin and eosin and the Van Gieson connective tissue stain. In addition sudan III stain for lipoid and the Von Kossa stain were used in the study of selected specimens. The histologic appearance of each valve was recorded on a plot sheet in a diagrammatic fashion so that a rough, somewhat quantitative analysis could be made of the data.

The earliest changes consisted in an alteration of the fibrocollagenous tissue on the aortic side of the angle of insertion of the valve leaflet. Normally this tissue consists of fairly closely packed, prominent bundles of fibrocollagenous tissue, which stain uniformly deep pink with eosin or the Van Gieson stain. A small number of fibroblasts with small, dark, elongated nuclei are present. In cases with minimal degrees of change there was a loss of staining power of the tissue and a breaking up of the fiber bundles into tiny, irregularly disposed fibrils giving a somewhat "frayed" appearance. This process initially occurred in numerous, small, adjacent, circumscribed foci which stood out in Van Gieson-stained preparations as pale, translucent areas. In more severely involved tissues these areas were confluent so that the process extended in a plane parallel to the overlying intima from the insertion point of the aorta elastica to the base of the valve leaflet, and out to involve the fibrosa of the leaflet itself. Occasionally isolated foci of change appeared near the tip of the leaflet without any connection with the process at the base of the valve. These prominent confluent areas contained no remnants of the original well defined bundles of fibrocollagenous tissue, and no nuclei of fibroblasts remained. This absence of nuclei was a striking feature and may indicate that the tissue was necrotic. Further alterations consisted in a deposition of fatty material in these areas, together with masses of somewhat colsely packed needle-shaped clefts or cavities located usually just beneath the endocardium. The area of collagenous change was sometimes almost completely filled with such fatty deposits. The clefts frequently replaced the fibrosa of the valve leaflet, where they usually lay perpendicular to the intimal surface. The next most frequent change was the presence of variable numbers of discrete, dark blue, spherical granules, varying in size from fine, dustlike particles to distinct bodies about 7 microns in diameter. Occasionally the granules had a somewhat cystic appearance with a pale central area and a thin, darker rim. They were present in most of the sections which displayed the previously described alterations of the collagenous tissue. They were usually dispersed but occasionally formed small masses or clumps. It is believed that these granules probably contained calcium, because in Von Kossa-stained sections they appeared as black particles. In the altered areas occasional large mononuclear cells were present. These usually possessed irregular, dark, prominent nuclei. Occasional macrophages were distended by numerous fine globules of stainable fatty material.

The alterations mentioned were associated with a variable degree of swelling and an increase in rigidity of this area which could be demonstrated in the gross specimen. This probably produced the frequent "ridging" and stiffening of the base of the valve described previously. In cross sections of the valve the swelling was easily seen (fig. 4).

Concomitant with the process described, a fairly constant amount of lipid is deposited within the intima of the sinus of Valsalva. This process also appeared to begin at the angle of insertion and to extend back toward the aorta and out onto the leaflet. Grossly, the deposits were sharply demarcated, pale yellow areas which resembled the similar deposits in the aorta. Histologically, they consisted of masses of macro-

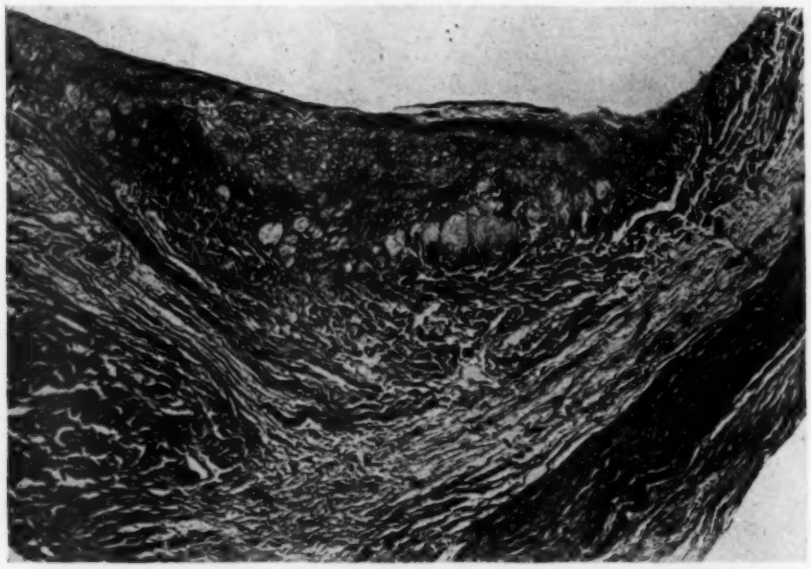

Fig. 4.—Close-up of the angle of insertion. Note cystlike areas of pale-staining tissue lacking structural detail and numerous acicular cavities indicating deposition of lipid. A slight degree of intimal thickening is also present. Van Gieson stain; × 59.

phages whose cytoplasm was distended with numerous discrete fine fat droplets which were readily stained with sudan III. Most of these cell clumps were located external to the deeper elastic layers of the intima. They were frequently separated by a fine reticulum of fibrous tissue within the intima.

It was in the areas of collagenous change at the angle of insertion that small discrete deposits of calcium were first found. Frequently they could be demonstrated only with the microscope. They were usually located just beneath the slightly thickened intima. It has not been possible to determine whether or not they arose as a result of the coalescence of

the numerous fine calcium granules previously described. Adjacent to the large calcium masses the granules were especially numerous and closely packed. The calcium itself appeared as bulky, irregular, sharply angular masses that frequently contained sharp acicular cavities and angular spaces. Surrounding each mass was usually an area of almost acellular hyaline collagenous tissue containing numerous cleftlike cavities and calcium granules. Larger calcium masses bulged into the sinus of Valsalva and were covered by a thin layer of fibrous tissue. In severe involvements the entire space was often filled by a bulky calcareous mass. The calcium occasionally extended into the fibrosa of the leaflet for a considerable distance, producing a distinct distortion of the valve (fig. 3*B*). The process always appeared to occur in the areas of altered collagenous tissue previously described. In the leaflet the calcium was limited to the fibrosa. Occasionally, isolated calcium flecks appeared in the outer portion of the leaflet near the tip, where areas of hyalinization of the fibrosa have been previously described. Occasionally, small flecks of bone possessing well defined structure and even occasional marrow elements were present in the larger calcium masses.

Inflammatory changes of a mild nature were occasionally found adjacent to the calcium deposits. They consisted in slight focal cellular infiltrations, the cells being lymphocytes and occasional mononuclears and eosinophils. Frequently young fibroblasts and small capillaries were seen. The fact that such changes were not present in similar areas free of calcium suggests that they may be related to the calcium mass itself.

Of 61 aortic valves not containing gross calcium masses, 59 possessed distinct areas of collagenous alteration. In 34 the process was present at the angle of insertion and extended distinctly into the base of the leaflet. In 18 there was only slight or no extension, the process being confined entirely to the region at the point of insertion. In 9, isolated, discrete areas of collagenous alteration were present well out in the fibrosa of the leaflet, unconnected with any process at the base. In every valve showing collagenous alterations discrete blue calcium granules were present as well. In 6 there were discrete masses of calcium which measured up to 1 mm. in diameter. These were not seen in roentgenograms of the valves and hence were not reported in the series of grossly demonstrable calcifications. The fact that these were encountered in single microscopic sections of valves in which their presence was not suspected after external examination indicates that a more careful microscopic search of serial sections would reveal a much higher incidence of such tiny deposits of calcium.

The high frequency of preexisting degenerative changes at the points of calcium deposition, the gradual transition from microscopic calcium flecks to bulky masses and the absence of signs of inflammation suggest

that the process is progressive, essentially degenerative in nature and similar to alterations seen elsewhere in the vascular system which accompany the aging process.

CLINICAL OBSERVATIONS

Clinically the patients whose aortic valves contained calcium deposits showed no constant manifestation of the disease until the deposits were so extensive as to cause a severe degree of stenosis of the aortic orifice. As pointed out previously, most of the patients were elderly. The incidence of the process appeared to be equally distributed between males and females. It was prevalent in the older age groups, and arteriosclerosis was often present elsewhere in these patients. Roentgenograms of the chest frequently revealed an elongated, tortuous aorta with visible calcium plaques. Peripheral arteriosclerosis was common. Hypertension, was however, not common. Of 32 patients with aortic calcifications, 5 had blood pressures above 160 systolic and 100 diastolic. The individual readings were 169 systolic and 100 diastolic, 160 systolic and 110 diastolic, 160 systolic and 102 diastolic, 180 systolic and 110 diastolic and 170 systolic and 100 diastolic. Most of the remaining patients had a mild systolic elevation of the type usually associated with arteriosclerosis of the larger vessels. In 17 of the 32 patients mentioned systolic murmurs were present before death. Although a high incidence of such murmurs is usually present in such an older age group, there was some indication that a part of the murmurs might be related to the calcium deposited in the valves. Separating the 32 patients with calcifications into two groups of 16—one possessing small calcium flecks only and the other possessing large, prominent calcium deposits—one finds that in the former group only six systolic murmurs were demonstrable. Two were classed as grade 3, three as grade 2 and one as grade 1. In the latter group, with the larger calcium depoists, eleven systolic murmurs were heard. One was classed as grade 4, three as grade 3, two as grade 2 and five as grade 1. Accurate data regarding the question of whether or not the murmurs were transmitted to the vessels of the neck are not available. It has been mentioned (Herrick[13]) that a systolic murmur heard in the aortic area may presage for many years a later sclerotic aortic stenosis.

COMMENT

The high incidence of grossly demonstrable calcifications of the aortic valve in hearts free from evidence of previous rheumatic disease has been demonstrated (34 per cent of 100 hearts observed at autopsies). Histologic examination of aortic valves in which gross calcium deposits

13. Herrick, J. B.: J. A. M. A. **101**:438, 1933.

were not demonstrable has revealed 6 instances in which microscopic calcium masses were present in single, unselected sections. Thus the total incidence of calcification of the aortic valve, gross and microscopic, is remarkably high in hearts not exhibiting evidence of rheumatic involvement. In addition the incidence of such calcification shows a gradual increase with advancing years. Finally, the size of the calcific deposits is greater in the older age groups. All transitions of the process can be found from the microscopic flecks of calcium observed in valves of a younger age group to the prominent, bulky calcium masses producing marked deformity of the aortic valve of the older age group.

These findings suggest that cases of full-blown aortic stenosis arise as a result of a similar process which is not necessarily associated with inflammatory heart disease and which parallels degenerative changes occurring with age elsewhere in the vascular system. The appearance suggests that the development of the process is gradual, but it is possible that there are repeated episodes of calcification following injury of tissue rather than continuous intravalvular precipitation of calcium. Signs of an inflammatory component, recent or old, are not recognizable in most of the cases.

It has been contended by Karsner and Koletsky[2] that in their series of 200 cases of aortic stenosis practically every case presented evidence of previous rheumatic involvement. A careful microscopic search, which revealed histologic lesions, often of a mild degree, in various regions of the heart, was necessary in many instances to obtain evidence that there had been previous inflammatory heart disease. The coexistence of such lesions and calcification of the aortic valve is at best only suggestive of an etiologic relationship. It must be noted that such histologic lesions are encountered in a majority of hearts studied in such a manner. Hall and Anderson[14] found such lesions in 90 per cent of 112 hearts free of gross lesions of chronic valvular disease. It is possible that rheumatic infection may produce the alterations in the collagenous tissue which precede the deposition of calcium, but this process has not been described. Yet there is apparently some relation between old rheumatic disease and calcification of heart valves, since the two occur together frequently. A possible explanation which would satisfy the observed facts is that rheumatic involvement accelerates a degenerative process which, however, can occur to some degree in many hearts in which there is no evidence of valvulitis. The distribution of the smaller lesions, which are almost all located at the base of the valve cusps where the maximum flexion of the tissue occurs during movement of the valve, suggests that mechanical factors may play a role, as has been postulated for atheromatous lesions. It is possible that the influence of rheumatic disease on the calcification

14. Hall, E. M., and Anderson L. R.: Am. Heart J. 25:64, 1943.

of the valve is mediated through a modification of the mechanical strains in thickened valves.

SUMMARY

In 100 consecutive, unselected autopsies of adults, calcium deposits were observed in the aortic valves in 46 hearts. In 34 of these hearts no gross or histologic evidence of valvular rheumatic disease was found.

Such calcium deposits were found more frequently in older persons, and their size and number were greater in the higher age groups.

Microscopic examination of the valves revealed that in most cases there were alterations of the dense connective tissue at the base of the leaflet and to a lesser degree in the fibrosa of the leaflet itself. These alterations resembled degenerative processes occurring elsewhere in the vascular system.

Calcifications were found most frequently in these areas, suggesting that the deposition of calcium is part of the same degenerative process.

The findings suggest that in many cases calcifications of the aortic valves are a result of a purely degenerative process and do not have a rheumatic origin.

INFLUENCE OF SYMPATHECTOMY ON THE NECROSIS DEVELOPING IN RABBITS' EARS AFTER THE SKIN HAS BEEN FROZEN WITH SOLID CARBON DIOXIDE

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SYMPATHECTOMY or sympathetic block has been advocated for the treatment of frostbite, trench foot and allied cold injuries by numerous investigators. Many of them, however, have given rather incomplete information as to the stage of the process treated and as to the concrete results. A critical control material is usually not presented, and the theoretic background for the argument pro or contra this intervention is usually based on rather vague evidence.

The data presented here constiute a preliminary report of experiments designed to show the influence of sympathectomy on the development of necrosis in rabbits' ears after the skin has been frozen with solid carbon dioxide.

Kreyberg and Rotnes[1] showed, in 1931, that when solid carbon dioxide was applied to the ears of rabbits for approximately three seconds stasis often developed within the capillaries of the skin, leading subsequently to necrosis.

In the present experiments the object was to find whether necrosis of the skin of the sympathectomized ear was either more or less severe, or earlier or later, than that appearing after similar cold injury of the opposite normal control.

Every experimental study of a comparative nature faces the problems of inconstancy of dosage and individual variations. Either large numbers of experimental animals are necessary, or one must use the same animals for experiment and for control.

TECHNIC

Operation.—The superior cervical sympathetic ganglion was removed from the right side of each rabbit, the animal being under ether narcosis. The success

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The experiments were carried out during my stay at the 108th United States General Hospital, Paris, in 1945 (Col. L. M. Rousselot, commanding officer).

1. Kreyberg, L., and Rotnes, P. L.: Compt. rend. Soc. de biol. **106**:895, 1931.

of the operation was recognized, after the animal had recovered from the effect of narcosis, by the increased dilatation of the blood vessels and the increased temperature of the skin of the ear. Sympathectomy was always performed on the right side, and the left side was used as a control.

Freezing.—This was done by applying a stick of solid carbon dioxide 9 mm. in diameter (formed by pressing the "snow" into a funnel) to the skin with even and constant pressure. The dosage was calculated to correspond to the threshold dosage for producing necrosis of the skin.

The degree of the exposure is tabulated as a symbol a/b, where a is the time, in seconds, during which carbon dioxide stick was applied to the skin (the period of application) and b is the time, in seconds, that elapsed from the moment of the application of the stick until thawing was complete (the total period of exposure). In the cases in which the symbols are identical, it is presumed that the dosages have been similar.

The fields to be frozen were selected under as nearly identical conditions as possible in the following areas: (1) near the tip of the ear, (2 and 3) in the body of the ear between the larger vessels, and (4) near the base of the ear. The fields were numbered 1, 2, 3 and 4, the numbering always beginning from the tip of the ear. As a rule, the freezing was done from the external side, except that near the base of the ear, where the fur is heavier, the carbon dioxide stick was applied to the inner surface of the ear.

Individual Variations.—The experimental rabbits varied in size, age, sex and color. Some were albinos and some white with faint gray spots. The nutritional state varied (France, 1945), and also the source of origin of the animals. Accordingly, the thickness of the ears proper and the fur varied not only from one field to another in the same ear but also in identical fields from one animal to another.

EXPERIMENTAL RESULTS

In figure 1 are recorded the period of application and the total period of exposure in seconds. The chart comprises the 5 animals from the special experiment (rabbits 5 to 9) and 4 animals from another experiment (rabbits 1 to 4). It shows:

1. With the degree of freezing used in this experiment (threshold dose), a longer period of application is followed by a longer peiod of thawing. This relationship will evidently not hold good for applications of much longer duration than those used in these experiments.
2. From the exact figures quoted in tables 1 to 5 one may conclude that the thawing proceeds slightly more rapidly in the middle of the ear (areas 2 and 3) than at the tip and at the base. This observation further emphasizes the necessity of comparing exactly identical fields of the two ears.
3. There is a marked individual difference in thawing time after the same period of application.
4. The period of thawing is usually shorter on the side of sympathectomy than on the side not operated on, but the difference

is not as great as the difference shown from one animal to another.

In the first few days the gross tissue reaction to the freezing is twofold: first, hyperemia and, second, development of edema. The animals were examined one, twenty-four and forty-eight hours after freezing, and later, every second day for two weeks.

In the tables the degree of hyperemia is recorded according to the degree of the red color of the frozen field; the degree of edema, according to the degree of the visible and the palpable swelling. The reactions were designated as "strong," "considerable," "some" and "light."

After the fourth day additional reactions were recorded, viz.: desquamation of the epidermis, oozing of fluid from the injured area, subsequent

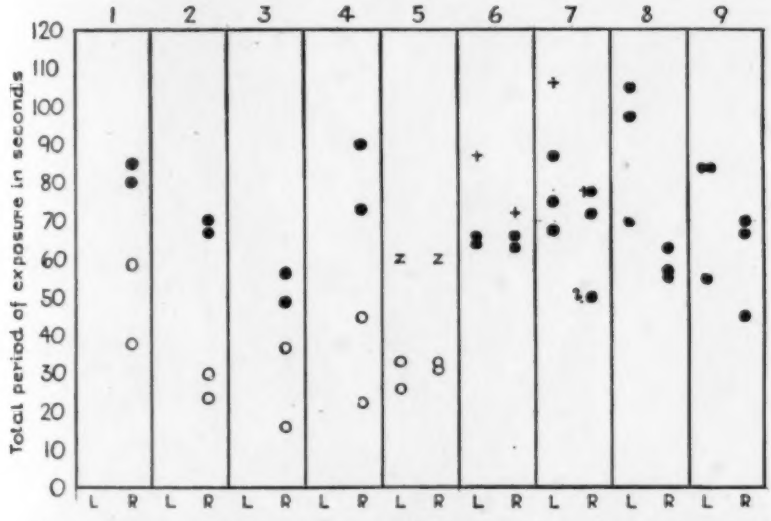

Fig. 1.—Record of the period of application of solid carbon dioxide and the total period of exposure of each ear of each rabbit. The duration of application is indicated as follows: 2 seconds by a white circle; 3 seconds by a black circle; 4 seconds by a cross; 5 seconds by the letter Z. The rabbits are numbered from 1 to 9 at the top of the chart. Prior to being exposed to cold injury, rabbits 5 to 9 had been treated by removal of the superior cervical sympathetic ganglion of the right side. *L* indicates the left ear; *R*, the right ear.

formation of crust and finally ulceration. The grading of these reactions is given as percentage area involved; i.e., 75 per cent indicates that three fourths of the frozen area was involved. The final result was in all cases a more or less pronounced scar.

After thawing, within a period of five minutes to one hour, the development of the reactions is more rapid on the sympathectomized side. The hyperemia may or may not be a little stronger, but the edema always develops faster and is stronger on that side. The edema may often

be seen as a yellowish cushion around the frozen field (fig. 2), partly obscuring the hyperemia.

In all cases the edema continues to be stronger on the symathectomized side, even if the total period of exposure was shorter than on the control side.

The development of oozing, crust formation, ulceration or desquamation is mainly influenced by the length of the period of exposure. In the cases in which the symbols of the right and left sides are identical, and

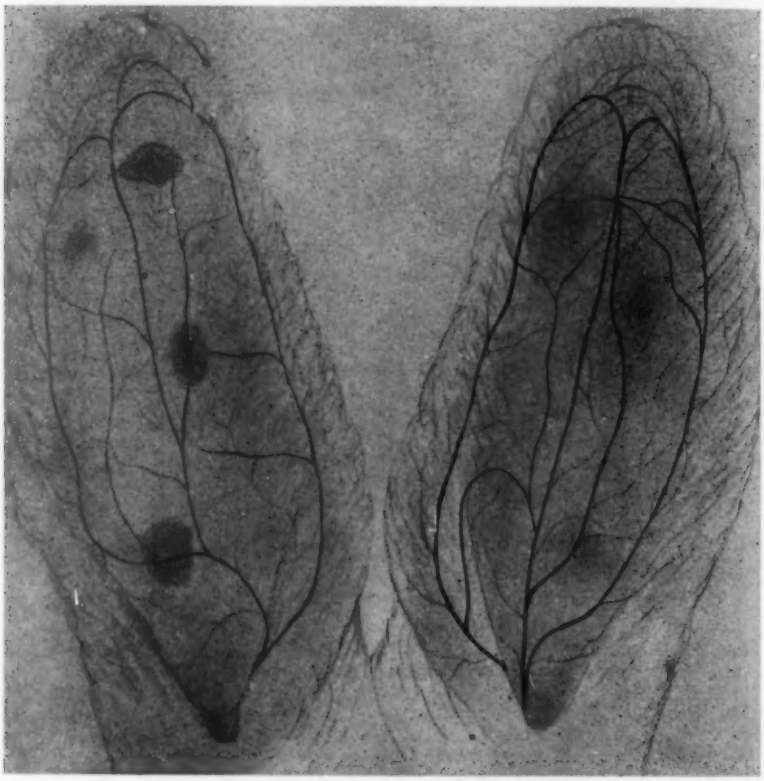

Fig. 2.—A reproduction in black and white of a colored sketch made from the ears of the living animal (rabbit 7) one hour after freezing.

therefore the total periods of exposure are presumably of the same order, the development is more rapid, also slightly more violent, on the sympathectomized side. The final result is not less favorable, in spite of the more violent reaction. In their main features these observations are in agreement with the observations made by Samuel[2] in 1890. Samuel ex-

2. Samuel, S.: Virchows Arch. f. path. Anat. **121**:396, 1890.

TABLE 1.—*Protocol of Rabbits 5 (White, Weighing 1,750 Gm.) Which Underwent Sympathectomy of the Right Side on March 24, 1945 and Freezing of Both Ears on March 25*

Field	Left Ear: (a) Period of Application and (b) Exposure a/b*	Left Ear: Reaction	Right Ear: (a) Period of Application and (b) Exposure a/b*	Right Ear: Reaction
		24 Hours		
1	2,5/38	Light hyperemia, no edema	2,5/52	Strong hyperemia, some edema
2	5/60	Considerable hyperemia, light edema	5/60	Considerable hyperemia, light edema
3	2/26	Light hyperemia, light edema	2/31	Some hyperemia, some edema
4	2/33	Some hyperemia, some edema	2/33	Some hyperemia, some edema
		4 Days		
1		Normal		Light desquamation, outside
2		Crust, 25%, outside		Desquamation, both sides
3		Light hyperemia		Some hyperemia
4		Strong desquamation inside		Crust, 100%, inside
		8 Days		
1		Normal		Crust, 75%, outside
2		Crust, 50%, both sides		Considerable desquamation both sides
3		Normal		Normal
4		Crust, 75%, inside		Crust, 75%, inside
		12 Days		
1		Normal		Desquamation, 25%, both sides
2		Crust, 50%, both sides		Desquamation, 25%, both sides
3		Normal		Normal
4		Ulcer, 75%, inside		Ulcer, 50%, inside

*In this and subsequent tables, the period of application as defined in the text under "Technic" is the number of seconds during which the solid carbon dioxide was applied, and the period of exposure is the number of seconds that elapsed from the time when solid carbon dioxide was applied to the time when thawing was complete. a/b is the symbol of the degree of exposure. Comment: Two pairs of corresponding fields (2 and 4 of both sides) had identical exposures, and one pair (3 of both sides), practically the same exposures. The initial reaction was identical in the fields with identical exposures. In the remaining two fields (1 of both sides) the initial reaction was slightly more intense when the exposure was prolonged. The latter reactions indicate a little more severe damage in the control ear, in the areas of equal exposure.

TABLE 2.—*Protocol of Rabbit 6 (White, Weighing ?) Which Underwent Sympathectomy of the Right Side on March 25, 1945 and Parallel Freezing of Both Ears on March 29*

Field	Left Ear		Right Ear	
	(a) Period of Application and (b) Exposure a/b*	Reaction	(a) Period of Application and (b) Exposure a/b*	Reaction
		24 Hours		
1	3/66	Strong hyperemia, no edema	3/66	Strong hyperemia, some edema
2	4/87	Strong hyperemia, some edema	4/72	Strong hyperemia, considerable edema
3	3/65	Considerable hyperemia, light edema	3/63	Strong hyperemia, strong edema
		4 Days		
1		Considerable hyperemia, no edema		Considerable hyperemia, some edema
2		Considerable hyperemia, some edema		Strong hyperemia, some edema
3		Some hyperemia, considerable edema		Strong hyperemia, considerable edema
		8 Days		
1		Considerable hyperemia, some edema		Desquamation outside
2		Considerable hyperemia, some edema		Oozing, 25%, outside
3		Considerable hyperemia, some edema		Strong hyperemia, considerable edema
		9 Days		
1		Considerable hyperemia, some edema		Oozing, 25%, outside
2		Desquamation outside		Oozing, 50%, outside
3		Considerable hyperemia, some edema		Desquamation

*This symbol is explained under table 1.
Comment: The exposures of fields 1 were identical; those of fields 3 were practically identical. The initial reaction was stronger on the sympathectomized side. On the ninth day the necrotic processes were also more advanced on the sympathectomized side. The premature death of the animal prevented comparison of the final results

TABLE 3.—*Protocol of Rabbit 7 (White, Weighing 2,150 Gm.) Which Underwent Sympathectomy of the Right Side on April 13, 1945 and Parallel Freezing of Both Ears on April 3*

Field	Left Ear		Right Ear	
	(a) Period of Application and (b) Exposure a/b*	Reaction	(a) Period of Application and (b) Exposure a/b*	Reaction
		24 Hours		
1	3/75	Considerable hyperemia, some edema	3(?)/50	Some hyperemia, some edema
2	3/68	Strong hyperemia, some edema	3/72	Considerable hyperemia, considerable edema
3	3/87	Strong hyperemia, some edema	3/78	Strong hyperemia, strong edema
4	4/106	Strong hyperemia, some edema	4/78	Strong hyperemia, strong edema
		4 Days		
1		Considerable hyperemia, considerable edema		Light hyperemia, considerable edema
2		Considerable hyperemia, considerable edema		Considerable hyperemia, considerable edema
3		Strong hyperemia, considerable edema		Strong hyperemia, considerable edema
4		Oozing, 25%, inside		Oozing, 75%, inside
		8 Days		
1		Desquamation outside, considerable edema		Desquamation, outside, light edema
2		Desquamation outside, some edema		Desquamation outside, some edema
3		Oozing, 75%, inside		Oozing, 100%, inside
4		Oozing, 100%, inside		Oozing, 100%, inside
		12 Days		
1		Some desquamation both sides		Light desquamation outside
2		Light desquamation outside		Desquamation outside, some edema
3		Crust, 75%, inside		Crust healing
4		Crust, 75%, inside		Crust, 75%, inside

*This symbol is explained under table 1.

Comment: The period of application of solid carbon dioxide was identical in both ears except possibly in fields 1; but the total period of exposure was reduced on the sympathectomized side, owing to a reduction of the thawing time. The initial reaction hyperemia was nearly the same, but there was considerably more edema on the sympathectomized side (fig. 2). When oozing of fluid began, it began earlier on the sympathectomized side, and it seems that healing was completed in a shorter time. The final and total result of the freezing seems to be practically identical on the two sides.

TABLE 4.—*Protocol of Rabbit 8 (White, Weighing 1,950 Gm.) Which Underwent Sympathectomy of the Right Side on April 4, 1945 and Parallel Freezing of Both Ears on April 4*

Field	Left Ear		Right Ear	
	(a) Period of Application and (b) Exposure a/b*	Reaction	(a) Period of Application and (b) Exposure a/b*	Reaction
		24 Hours		
1	3/105	Strong hyperemia, considerable edema	3/63	Some hyperemia, considerable edema
2	3/70	Light hyperemia, some edema	3/56	Light hyperemia, considerable edema
3	3/96	Some hyperemia, considerable edema	3/57	Light hyperemia, strong edema
4	3/?	Considerable hyperemia, Considerable edema	3/?	Light hyperemia, strong edema
		4 Days		
1		Strong hyperemia, no edema		Considerable hyperemia, considerable edema
2		Light hyperemia, no edema		Light hyperemia, no edema
3		Considerable hyperemia, considerable edema		Light hyperemia, some edema
4		Considerable hyperemia, considerable edema		? hyperemia, no edema
		8 Days		
1		Strong hyperemia, considerable edema		Some hyperemia, some edema
2		Light hyperemia, no edema		Light hyperemia, no edema
3		Considerable hyperemia, considerable edema		Light hyperemia, no edema
		12 Days		
1		Desquamation outside		Normal
2		Normal		Light hyperemia, no edema
3		Crust, 25%, outside		Normal
4		Light hyperemia, light edema		Normal

*This symbol is explained under table 1.

Comment: The period of application of solid carbon dioxide was the same on both sides; the total period of exposure was definitely shorter on the sympathectomized side, owing to a reduction of the thawing time. The initial reaction showed less hyperemia but more edema on the sympathectomized side. The later reactions were more severe on the control side; the hyperemia and edema were more persistent, and the destructive changes were more severe. The different reactions on the two sides were proportional to the actual periods of exposure.

TABLE 5.—*Protocol of Rabbit 9 (White, Weighing 1,750 Gm.) Which Underwent Sympathectomy of the Right Side on April 4, 1945 and Parallel Freezing of Both Ears on April 4.*

Field	Left Ear		Right Ear	
	(a) Period of Application and (b) Exposure a/b*	Reaction	(a) Period of Application and (b) Exposure a/b*	Reaction
		24 Hours		
1	3/55	Light hyperemia, light elema	3/45	? hyperemia, light edema
2	3/84	Strong hyperemia, some edema	3/70	Strong hyperemia, strong edema
3	3/84	Strong hyperemia, some edema	3/67	Strong hyperemia, strong edema
		4 Days		
1		Light hyperemia, no edema		Normal
2		Strong hyperemia, considerable edema		Strong hyperemia, considerable edema
3		Strong edema strong edema		Strong hyperemia, strong edema
		8 Days		
1		Normal		Normal
2		Oozing, 50%, outside		Light desquamation outside
3		Oozing, 25%, inside		Desquamation inside
		12 Days		
1		Normal		Normal
2		Crust, 50%, outside		Light desquamation outside
3		Crust, 50%, inside		Light desquamation inside

*This symbol is explained under table 1.
Comment: The period of application was the same on both sides, but the total period of exposure, because of more rapid thawing, was reduced on the sympathectomized side. Initially the edema was stronger on the sympathectomized side. The total reaction and the destruction were definitely stronger on the control side, corresponding to the longer periods of exposure to cold.

amined the reactions to heat in the sympathectomized and the opposite ear. His experiments with heat (water at 54 C.) and the present experiments with cold suffer from one important experimental error, which I have not seen mentioned, viz., the cooling and the heating effect of the blood vessels of the exposed area. In the sympathectomized ear in a series of the present experiments, the increasd circulation definitely shortened the period of exposure. This may account to some extent for the lesser damage of the tissue of the sympathectomized ear. A similar condition may account for the lesser damage done by the scalding in Samuel's experiments. After this difference has been accounted for, there is still a certain lessening of the damage, which may be explained by the change of the blood flow proper—and I accept the explanation of Samuel as being valid also for the damage due to cold—namely, the partial prevention of stasis. The widely different results when roentgen rays are used to produce the damage, as shown by Natvig,[6] in 1936, in my laboratory, may be a key to a better understanding of the fundamentals of the development of these injuries.

SUMMARY

The vascular reactions of the sympathectomized ear to freezing follow a different course from those of the opposite (control) ear. The difference is manifested by a more rapid and more abundant development of edema. The tissue reactions vary in different rabbits and in different areas of the same ear. Whether the local changes will lead to necrosis of the tissues of the frozen area is, with the doses used in this experiment, decided mainly by the degree of the local insult. The tissue reactions are slightly more violent on the sympathectomized side, but the final damage is not altered, as measured with the present technic.

3. Natvig, P.: Acta path. et microbiol. Scandinav., 1936, supp. 26, p. 239.

EFFECTS OF ELECTROLYTE IMBALANCE ON THE ADRENAL GLAND

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IT IS WELL KNOWN that the adrenal gland is an important factor controlling the electrolyte balance in the body. When the adrenal cortex atrophies or is removed, the sodium content of the body decreases and the potassium content increases. This is a major disturbance in Addison's disease (hypofunction of the adrenal cortex due to tuberculosis, or to an undetermined disease of the adrenal gland).

Many studies have been made on the amounts of sodium and potassium required by the normal body. These studies have neglected the histologic aspects of the adrenal gland; however, there are a few recorded observations. Schrader, Prickett and Salmon[1] and Kornberg and Endicott[2] noticed anatomic changes in the adrenal gland resulting from a potassium-deficient diet. Orent-Keils, Robinson and McCollum[3] reported changes occurring in the adrenal gland when a sodium-deficient diet was administered. These observations were incidental to biochemical studies. The experiments described in this report represent an attempt to ascertain these changes more precisely.

MATERIALS AND METHODS

Sixty 150 Gm. male albino rats of the Wistar strain were used in this experiment. Ten rats were maintained on a Purina laboratory chow; these animals were considered as normal. Twenty rats were maintained on a potassium-deficient diet. Twenty rats were maintained on a sodium-free diet. The remaining 10 animals were maintained on a diet prepared in the same manner but with both sodium and potassium present; these animals were designated as controls.

The diet used closely resembles that of Orent-Keils, Robinson and McCollum.[3] It was made up of chemically pure materials as follows. Sodium-deficient diet:

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1. Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: J. Nutrition 11: 85, 1937.

2. Kornberg, A., and Endicott, K. M.: Am. J. Physiol. 145:291, 1945.

3. Orent-Keils, E.; Robinson, A., and McCollum, E. B.: Am. J. Physiol. 119:651, 1937.

Acid-washed casein	4 lb.
Commercial sucrose	15 lb.
Acid-washed corn oil	1 pint
Calcium chloride ($CaCl_2$)	225 Gm.
Potassium chloride (KCl)	112 Gm.
Magnesium oxide (MgO)	22 Gm.
Potassium dihydrogen phosphate (KH_2PO_4)	168 Gm.
Ferric ammonium sulfate ($FeNH_4[SO_4]_2$	56 Gm.
Copper sulfate ($CuSO_4$)	11 Gm.
Cod liver oil	75 cc.
Thiamine	0.02 Gm.
Riboflavin	0.05 Gm.
Pyridoxine	0.02 Gm.
Pantothenic acid	0.02 Gm.
Nicotinic acid	0.50 Gm.

The whole lot was thoroughly mixed in a large stainless steel kettle and run through a meat grinder several times. Extreme care was taken to prevent its being contaminated with sweat. A diet prepared by Orent-Keils, Robinson and McCollum[3] in a similar manner was found to contain 0.002 per cent sodium. The control diet was prepared by adding 200 Gm. of sodium chloride to the sodium-deficent diet. The potassium-deficient diet was prepared by substituting sodium for potassium in the inorganic chemicals.

The animals were kept in groups of 5 in screen-bottomed cages. An excess of food was kept in "non-scatter" cups at all times. Distilled water was always available.

On the thirty-fifth day after the experiment had been started 1 of the potassium-deficient animals died; the rest of the potassium-deficient animals were killed with chloroform at this time. On the sixtieth day of the experiment 1 of the sodium-deficient animals died; all of the other sodium-deficient animals were killed with chloroform at this time. The normal rats and the control rats were also killed with chloroform on the sixtieth day.

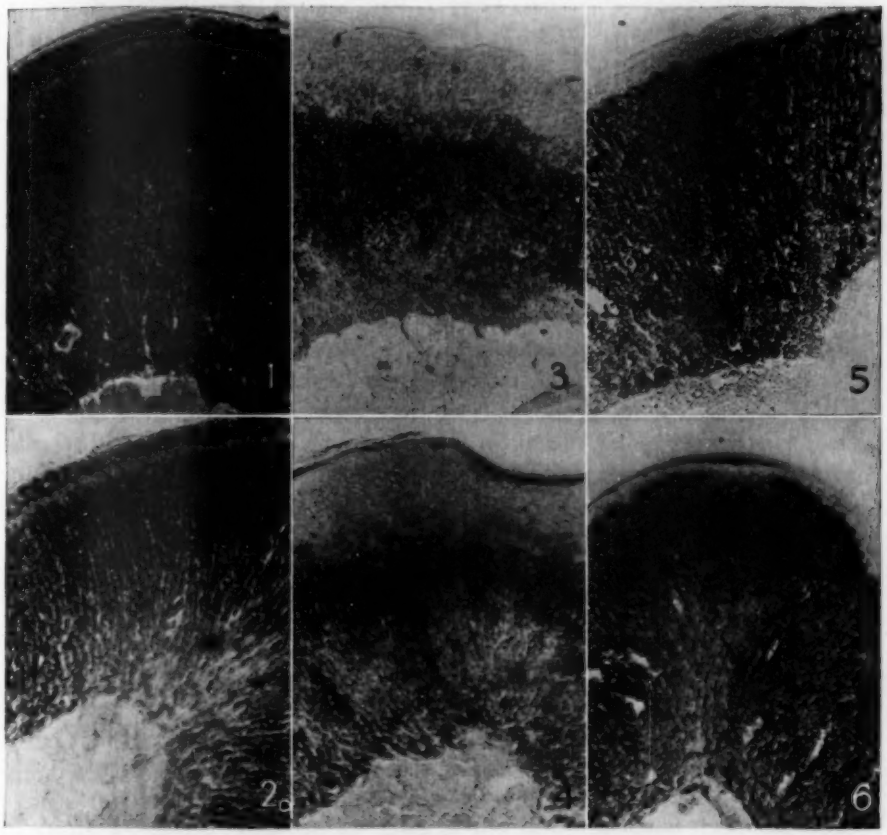
As soon as the animals died or were killed the adrenal glands were immediately removed and placed in a 1:10 dilution of formaldehyde solution U. S. P. for fixation. After twenty-four hours they were removed from the formaldehyde solution, and the surrounding fat was carefully dissected away. They were embedded in gelatin, sectioned at 10 microns thickness by the freezing method and mounted according to the method of Zwemer.[4] They were stained with sudan III by the method of Romeis[5] for the demonstration of total lipids. The Schultz reaction was used for the demonstration of cholesterols.[6]

RESULTS

The histologic observations will now be described. The distribution of the total lipids shown by the sudan strain on the adrenal glands of the normal animals is presented in figure 1; the distribution of the cholesterol fraction is shown in figure 2. The distribution of total lipids in the adrenal glands of sodium-depleted animals is shown in figure 3; here one sees marked reduction of lipids in the zona glomerulosa and the outer part of the zona fasiculata. The distribution of the cholesterol fraction revealed by the Schultz reaction, figure 4, closely follows that of the total lipids. The effect of the potassium-deficient diet on the adrenal glands is

4. Zwemer, R. L.: Anat. Rec. **57**:41, 1933.
5. Romeis, B.: Ztschr. f. mikr.-anat. Forsch. **16**:525, 1929.
6. Whitehead, R.: J. Path. & Bact. **39**:443, 1934.

shown in figures 5 and 6. Figure 5 indicates that the total lipids are depleted in the zona glomerulosa; figure 6, that the cholesterol fraction is also depleted in the zona glomerulosa in the same manner as the total lipids. The adrenal glands of the control animals were similar to those of the normal animals.



All the figures were taken at 135 diameters' magnification and reduced to 64.5 by the engraver. Figures 1, 3 and 5 show sudan-stained material; exposure 1/10 second with green Wratten filter no. 58. Figures 2, 4 and 6 show Schultz-stained material; exposure 1/5 second with red Wratten filter no. 29.

Fig. 1.—Normal adrenal gland. Note the normal distribution of total lipids.

Fig. 2.—Normal adrenal gland. Note the normal distribution of cholesterols.

Fig. 3.—Adrenal gland of a sodium-depleted animal. Note the depletion of lipids in the zona glomerulosa and the outer part of the zona fasiculata.

Fig. 4.—Adrenal gland of a sodium-depleted animal. Note that the cholesterols closely follow the total lipids.

Fig. 5.—Adrenal gland of a potassium-depleted animal. Note the depletion of total lipids in the zona glomerulosa.

Fig. 6.—Adrenal gland of a potassium-depleted animal. Note that the cholesterols closely follow the total lipids.

COMMENT

It has been noted that changes occur in the adrenal gland after administration of a diet deficient in sodium or in potassium; this is not surprising since the adrenal gland is known to have a profound influence on the metabolism of these substances. Kornberg and Endicott,[2] using a potassium-deficient diet, found that the adrenal gland showed "hydropic" changes. Schrader, Prickett and Salmon,[1] also using a potassium-deficient diet, found marked congestion of the adrenal glands. They also noted that the cortical cells stained poorly and appeared rarefied and edematous. Orent-Keils, Robinson and McCollum,[3] using a sodium-deficient died, reported: "The adrenals were orange in color and contrasted with the pale pink of the controls; they were smaller in every case. . . . The change in color of the adrenals is especially noteworthy." There have been no further observations or explanations.

In view of the close chemical relation of the cholesterols of the adrenal gland and the hormones elaborated by the gland it is assumed that the hormones are formed from the cholesterols. Levine,[7] Darrow and Sarason,[8] Dalton and co-workers[9] and others have shown that in anoxia there is marked depletion of the cholesterols, especially in the inner zones of the gland. This is true with many kinds of physiologic stress. Concurrent with this, Hoagland[10] observed an increase in the urinary output of 17-ketosteroids; he considered the adrenal gland as the major source of these compounds. From this one would expect to find depletion of cholesterols in the area of the gland which was suddenly called on to produce a hormone. This is the case in the experiments reported here.

It has been demonstrated by Smith,[11] Crooke and Gilmour,[12] Sarason[13] and others that the zona glomerulosa remains intact in the hypophysectomized animal, while the inner zones atrophy; in such an animal the sodium-potassium balance is maintained, indicating that the secretion of the glomerular zone is adequate for maintaining the electrolyte balance. Sarason[13] and others have shown that in animals which have been given injections of desoxycorticosterone, which is a potent sodium-retaining agent, there is atrophy of, and depletion of, lipids in the glomerular zone. From these two lines of evidence one might suspect that the zona glomerulosa regulates the electrolyte metabolism.

7. Levine, L.: Endocrinology **37**:34, 1945.
8. Darrow, D. C., and Sarason, E. L.: J. Clin. Investigation **23**:11, 1944.
9. Dalton, A. J.; Mitchell, E. R.; Jones, B. F., and Peters, V. P.: J. Nat. Cancer Inst. **4**:527, 1944.
10. Hoagland, H.: J. Aviation Med. **18**:450, 1947.
11. Smith, P. E.: Am. J. Anat. **45**:205, 1930.
12. Crooke, A. C., and Gilmour, J. R.: J. Path. & Bact. **47**:525, 1938.
13. Sarason, E. L.: Arch. Path. **35**:373, 1943.

The observations presented here support this theory in general. When there is a great need for sodium-retaining hormone, because of low intake of sodium, the cholesterols of the zona glomerulosa and outer zona fasciculata become depleted, since the excess hormone formation uses up the reserve cholesterols. This evidence indicates that the sodium-retaining hormone is produced in these zones.

The findings in the potassium-depleted animals are more difficult to explain if one follows the current concept that the concentration of the interstitial and plasma potassium is maintained through an antagonistic effect of the sodium concentration. Perhaps the glomerular zone secretes a potassium excretion factor (Fenn[14]).

SUMMARY

Evidence is presented which indicates that the outer zones of the adrenal gland are closely connected with the regulation of electrolyte balance.

14. Fenn, W. O.: Physiol. Rev. **20**:377, 1940.

EFFECT OF 20-METHYLCHOLANTHRENE ON THE TRANSPLANTABILITY OF SKIN OF MICE

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IN THE SKIN of mice painted with carcinogenic polycyclic hydrocarbons previous to the making of a wound, the epidermal proliferation was increased, but the tendency of the regenerating epithelium to migrate over the defect was not commensurate with the intensification of proliferation.[1] As possible causes of the decline of migration we considered alterations of the epithelium itself or of the wound base.

In order to analyze further the mechanism underlying this change, we extended our experiments and are now reporting on the effect of applications of 20-methylcholanthrene on the transplantability of the skin of mice.

MATERIAL AND METHODS

One hundred and seventy male and female mice of the strain RFi were used. They were 4 to 6 months old and were maintained on a standard diet of Purina laboratory chow and water. Two hundred and twenty-eight autogenous and 112 homoiogenous transplantations were made. The following groups of experiments, illustrated in figure 1, were carried out:

Autotransplants.

Series 1.—Untreated skin was transplanted into a wound made in untreated skin.

Series 2.—Untreated skin was transplanted into a wound made in skin previously treated with 20-methylcholanthrene for six weeks.[2]

Series 3.—Skin painted with 20-methylcholanthrene for six weeks was transplanted into a wound made in untreated skin.

From the Snodgras Laboratory of Pathology, City Hospital.

This investigation was supported by a research grant from the National Cancer Institute of the National Institute of Health, United States Public Health Service.

1. Silberberg, M., and Silberberg, R.: Am. J. Path. **20**:809, 1944; Arch. Path. **42**:193, 1946.

2. A six week period of painting was considered most suitable for obtaining increased epithelial growth with a minimum number of tumors that might interfere with the grafting.

Series 4.—Skin painted with 20-methylcholanthrene for six weeks was transplanted into a wound made in skin likewise painted with this carcinogen for the same length of time.

Series 5.—Untreated skin was transplanted into a subcutaneous pocket made under skin painted previously with 20-methylcholanthrene for one-half, one, two or three months.[3].

Series 6.—Skin painted with 20-methylcholanthrene for one half, one, two or three months was transplanted into a pocket made under untreated skin.

Homoiotransplants.

Series 7 *to* 10.—The series of homoiotransplants (7 to 10) corresponded to series 1 to 4 with the modification that skin was taken from one animal and grafted into another animal of the same strain.

Animals of series 1 and 7 remained untreated previous to the skin grafting. Animals of series 2, 3, 5, 6, 8 and 9 had the lumbar region painted with a 0.3 per cent solution of 20-methylcholanthrene dissolved in benzene. Those of series 4 and 10 had the carcinogen applied to the entire length of the back. The carcinogen was applied three times weekly, each application being made with a single stroke of a camel's hair brush, no. 6, on a carefully clipped area of the skin.

As seen from figure 1, each mouse was carrying two transplants: In the case of autogenous grafts the transplants from the shoulder and lumbar regions were exchanged; in the case of homoiotransplants the skin from the shoulder or the lumbar region of one animal was grafted into the corresponding region of the other animal.

In those cases in which epilation had not yet taken place, the portions of skin to be grafted and the areas receiving the transplants were carefully shaved. A circular piece of skin measuring 4 mm. in diameter was removed with a pair of curved scissors from an area between the shoulder blades and from another area in the lumbar region. After excision of the pieces to be grafted, the margin of the wounds retracted, and the denuded areas were thus larger than the excised pieces of tissue. Each transplant was then attached with two silk sutures to one side of the margin of the wound to which it was transferred. In the case of subcutaneous transplants the excised tissue was transferred into a subcutaneous pocket, which was closed with silk sutures and sealed with collodion. Further details of the number of animals used and the number of transplants made in each group can be found in tables 1 to 7.

In series 1 to 4 and 7 to 10 the transplants were allowed to remain in situ for one, two, three, five, seven, ten or fourteen days; in series 5 and 6, for two, four, seven, ten or sixteen days. At the end of the experiments the grafts, with some surrounding and underlying tissue, were removed as a whole, fixed in formaldehyde solution U. S. P. (diluted 1:10) and embedded in paraffin, and serial sections were made and stained with hematoxylin and eosin.

HISTOLOGIC EXAMINATION

The main histologic observations and their incidence are given in tables 1 to 7. They relate to the number of living grafts, the proliferation and migration of the epithelium within the transplant, the outgrowing of the grafted epithelium toward the margin of the wound, and the time of closure of the wounds.

3. In groups 5 and 6 the periods of painting were the same as in our previous experiments on healing of wounds. Longer periods of painting could be chosen because tumors appearing on the skin overlying the transplants would not interfere with the growth processes of the subcutaneous transplants.

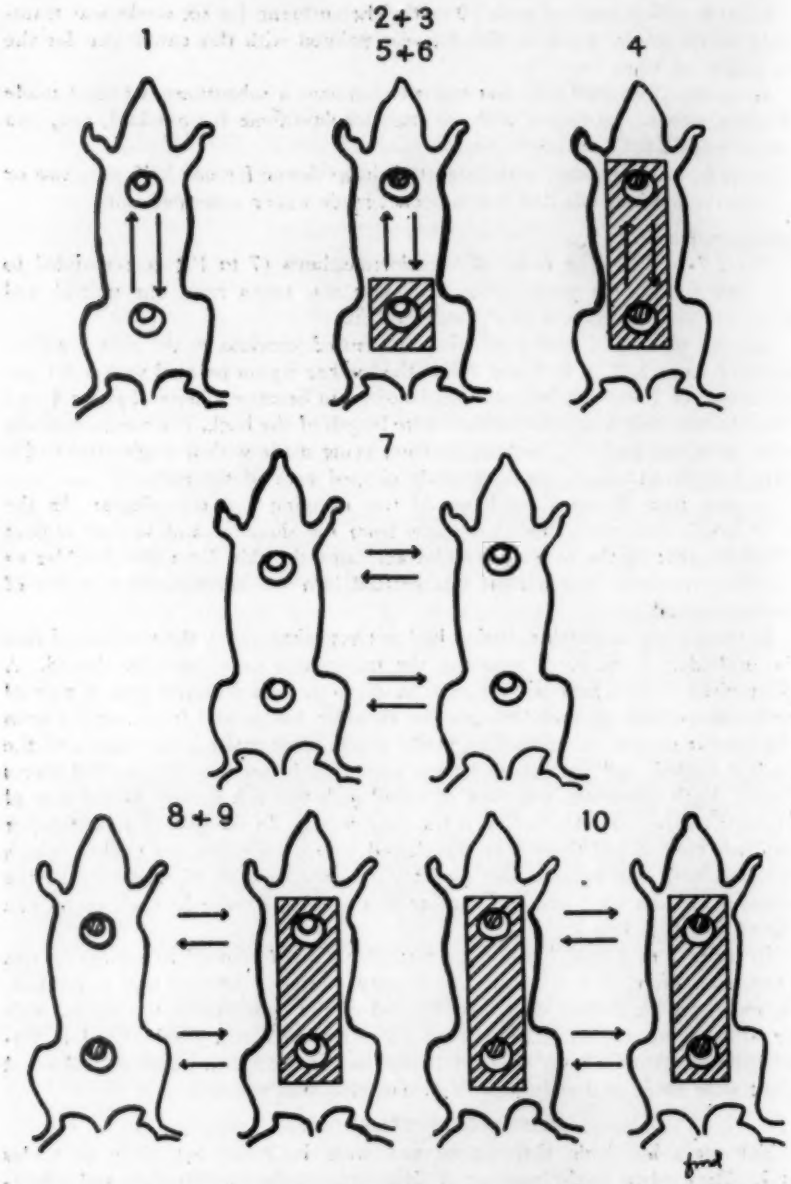

Fig. 1.—Diagramatic demonstration of the arrangement of the experiments. The number over each mouse or each pair of mice correspond to the serial number in the text. The shading indicates that these areas were painted with methylcholanthrene. The larger outer circles represent the wounds; the smaller inner ones, the grafts sutured eccentrically to one side of the wound margin. Arrows point away from the area from which the graft was taken and toward the recipient area of the skin.

Autotransplants.

Series 1: Untreated Skin Was Transplanted into a Wound Made in Untreated Skin.—The epithelium forming the margin of the wound was thickened, and the number of mitoses in the proliferating cells was increased. The intensity of these growth processes depended on the apposition of the graft. In those instances in which the transplant had retracted from the wound margin or was being sloughed off, the epithelium surrounding the defect was composed of as many as seven layers of cells, instead of the usual two or three, and contained from six to seven times the normal number of mitoses. Under these conditions the course of repair was similar to that seen in wounds without grafts as previously described.[1] The closer the contact of the graft and the margin of the wound, the less accentuated was the hyperplasia of the epidermis and the sooner it returned to the normal state. The peak of the marginal growth activity was usually seen three days after the transplantation. The marginal epithelium then consisted of four or five layers of cells, and the mitotic counts were increased four or five times. The proliferating epithelium migrated toward the graft, forming a tonguelike

TABLE 1.—*Autogenous Grafts: Untreated Skin Transplanted Into Wounds Made in Untreated Skin*

Days After Transplantation	Grafts Made	Grafts Living	Main Observations		
			Proliferation of Epithelium of Graft, Cases	Migration of Epithelium in Graft, Cases	Outgrowth of Epithelium from Graft, Cases
1	8	4	4	4	4
2	8	5	5	5	4 (1)†
3	8	6	6	6	3 (3)†
5	8	5 (1)*	5	5	5 (5)†
7	8	6 (7)*	6	4	6 (6)†
10	8	6 (8)*	6	2	6 (6)†
14	4	2 (4)*	2	0	2 (2)†
Total	52	34 (20)*	34	26	30 (23)†

*The number in parenthesis indicates the number of cases in which the wound had closed.
†The number in parenthesis indicates the number of cases in which the epithelium growing from the transplant had joined with the epithelial "tongue" growing out from the margin of the wound.

projection. After making contact with the transplant the epithelial "tongue" often split into two branches, one joining the surface epithelium of the graft and the other enveloping its hair follicles.

Thirty-four of the 52 autotransplants were living at the time of their removal (table 1.) In these instances the degree of proliferation and of migration of their epithelium varied. As a rule, the epithelium of the hair follicles remained better preserved and persisted for a longer period than the surface epithelium, and in the latter, again, the basal cells were more resistant than the spinous cells. One or two days after transplantation, the keratinized surface layer was found detached from the rest of the graft. The basal cells began to grow toward the margin of the wound in a tonguelike fashion (fig. 2*A*, *B* and *C*). The closer the contact established between the transplant and the surrounding epidermis, the earlier the epithelial "tongues" growing out from the graft and from the margin of the wound met. This occurred usually two or three days after transplantation (fig. 2*D*). Some parts of the grafts underwent atrophy or died. However, the

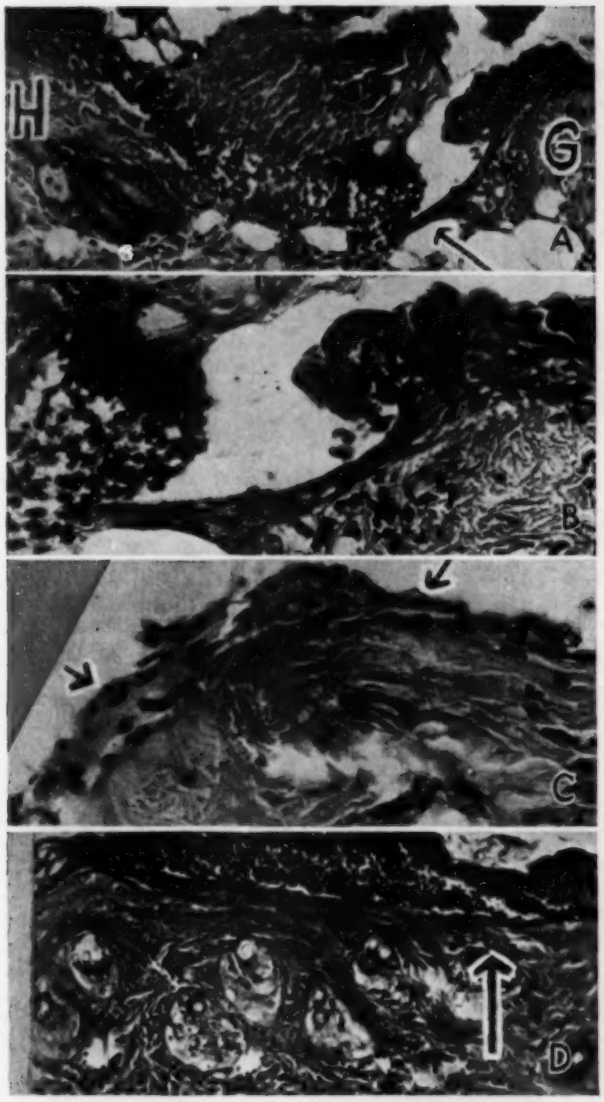

Fig. 2.—Autotransplants: In each instance, untreated skin grafted into wound made in untreated skin. *A* shows autotransplant (*G*) one day after it had been grafted into a wound made in untreated skin (*H*); × 190. An arrow points at epithelium growing out from the graft.

B, same as *A*. The outgrowing epithelium is shown under high magnification; × 475.

C, autotransplant two days after grafting; × 475. Epithelium is growing out from the surface of the graft.

D, autotransplant three days after grafting; × 190. Union of the epithelium of the graft and the epithelium of the wound has occurred. An arrow points at the site of union.

living epithelium proliferated and replaced the degenerated epithelium of the adjoining areas. This regenerative activity was particularly noticeable in the depths of the hair follicles from which epithelial "tongues" grew out (fig. 3). Here and there a slight infiltration of mononuclear leukocytes was noted in the wound base. After seven days all wounds were about to close or had closed, and the surviving grafts had been incorporated into the healed wound.

Eighteen autotransplants that had undergone atrophy were cast off by the newly grown epithelium advancing from the margin of the wound. In a few of

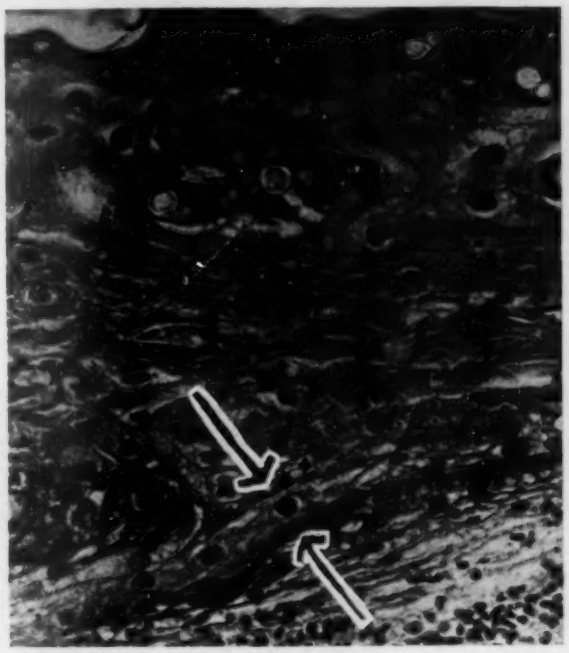

Fig. 3.—Autotransplant (untreated skin grafted into a wound made in untreated skin) three days after grafting; × 475. An arrow points at epithelium growing out from a regenerating follicle of the transplant. The epithelium in the upper part of the picture is that of the wound margin.

these wounds, there was no reaction in the base, and the regenerating epithelium grew over the defect in an almost straight line, as if there had been no transplant. In these cases there had apparently not been any contact between graft and wound base. In the majority of the cases in which the grafting was unsuccessful, edema and cellular infiltration of the wound base were noted, and the repair of the defect was slower than that of wounds showing no reaction.

Series 2: Untreated Skin Was Transplanted into a Wound Made in Skin Previously Treated with 20-Methylcholanthrene.—The painted epidermis was thickened, and the number of cell rows had increased from two or three to six. Keratinization of the spinous cells was intensified. The basal cells were enlarged, and the number of mitoses was increased fourfold to sixfold. The hair follicles were dilated and filled with keratin, and the sebaceous glands were frequently destroyed. In about one fourth of the animals mast cells had appeared in large

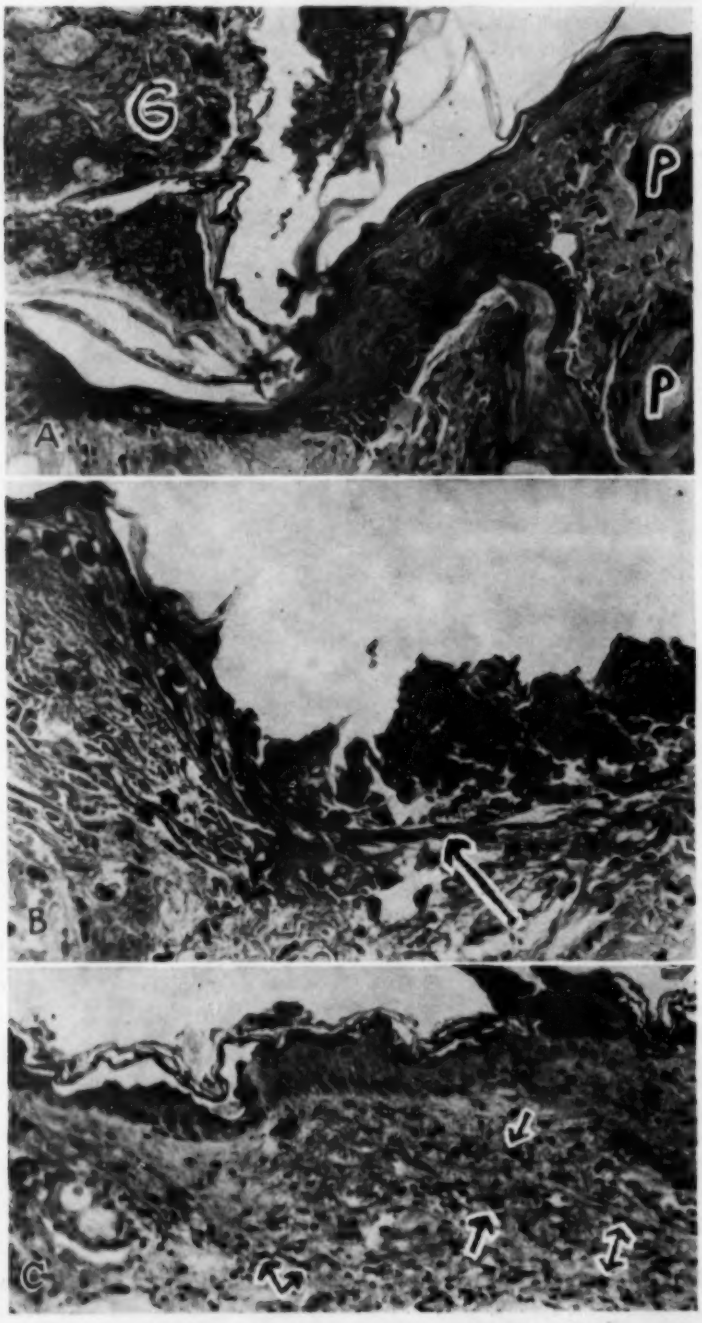

Fig. 4.—*A*, autotransplant (untreated skin grafted into a wound made in painted skin) five days after grafting; × 190. The wound is closed, the hyperplastic epithelium showing deep keratinizing pegs (*p*). The transplant (*G*) is being cast off.

numbers in the corium. The cutaneous collagen was fragmented in increased in amount. The subcutaneous tissue was loose and contained numerous congested capillaries.

After two days the growth processes at the line of excision were intensified: The number of mitoses had reached a peak of eight times the normal, and eight to ten layers of epithelium were piled up on top of each other. Simultaneously, enlarged and mitotically proliferating basal cells began to migrate toward the graft. As soon as they had reached it, the mitotic activity of the epithelium declined to three or four times the normal. This occurred between the third and the fifth day after grafting, depending on the better or the poorer apposition of the transplant. The regenerating epithelium either moved along the epidermal

TABLE 2.—*Autogenous Grafts: Normal Skin Transplanted Into a Wound Made in Skin Painted with 20-Methylcholanthrene*

Days After Transplantation	Grafts Made	Grafts Living	Main Observations		
			Proliferation of Epithelium of Graft, Cases	Migration of Epithelium in Graft, Cases	Outgrowth of Epithelium from Graft, Cases
1	4	3	3	3	2 (1)†
2	4	4	4	4	3 (1)†
3	4	3	3	3	1 (1)†
5	4	3	3	3	2 (2)†
7	4	1 (4)*	1	1	0
10	4	0 (4)*	0	0	0
14	2	0 (2)*	0	0	0
Total	26	14 (10)*	14	14	8 (5)†

*The number in parenthesis indicates the number of cases in which the wound had closed.
†The number in parenthesis indicates the number of cases in which the epithelium growing from the transplant had joined with the epithelial tongue growing out from the margin of the wound.

surface of the transplant or enveloped the hair follicles. In 12 cases the marginal epithelium did not make contact with the graft but, instead, invaginated into the cutis, forming epithelial pegs and keratin pearls (fig. 4*A*).

This occurred only on that side of the wound where the graft was attached. In these instances the mitotic activity remained high until the wound closed. Not infrequently, edema and cellular infiltration were noted in the wound base.

In the specimens taken out during the first seven days (table 2), 14 transplants were at least partly alive, and many contained mast cells that had migrated from the wound base. During the first two days after transplantation, the mitotic proliferation of the grafted epithelium went on. Again, the follicular epithelium was the better preserved, and it grew better than that of the surface, although there was atrophy in both. Also, the living epithelium migrated to some degree into those areas where the epithelium was dead. In 8 of these 14

(*Legend continued from Fig. 4*)

B, autotransplant (untreated skin grafted into a wound made in painted skin) one day after grafting; × 475. Epithelium is growing out from the unpainted graft. An arrow points at the outermost cell of the epithelium.

C, autotransplant (painted skin grafted into a wound made in normal skin) five days after grafting; × 190. The transplant is being cast off. Numerous mast cells are present in the cutis of the wound base (arrows).

living grafts the surface epithelium grew out centrifugally in the direction of the margin of the wound (fig. 4 *B*). In 2 transplants one and two days old, the outgrowing "tongues" began to merge with the nearby epithelium of the wound margin. After two or more days, wider areas of the transplants underwent regressive change and were infiltrated by mononuclear and polymorphonuclear leukocytes. Of the 10 grafts observed for seven or more days, 1 was incorporated into the healing wound; the others had been cast off in their entirety.

Series 3: *Skin Painted with* 20-*Methylcholanthrene Was Transplanted into a Wound Made in Untreated Skin.*—The mitotic proliferation of the marginal epithelium increased rapidly after the operation. It reached a maximum of five times the normal after two days; thereafter there was a continuous decline of the miotic counts. The epithelial "tongues" advancing with great rapidity from the margin of the wound undermined the graft. In these cases closure of the wound occurred after seven days. One or two days after transplantation the wound base was slightly infiltrated by mononuclear leukocytes. Subsequently fibroblasts ap-

TABLE 3.—*Autogenous Grafts: Skin Painted with* 20-*Methylcholanthrene and Transplanted into a Wound Made in Untreated Skin*

Days After Transplantation	Grafts Made	Grafts Living	Main Observations		
			Proliferation of Epithelium of Graft, Cases	Migration of Epithelium in Graft, Cases	Outgrowth of Epithelium from Graft, Cases
1	4	2	2	0	0
2	4	3	3	3	0
3	4	3	3	2	0
5	4†	3	3	3	0
7	4	3	3	3	0
10	4	2 (4)*	2	2	0
14	2	1 (2)*	1	1	0
Total	26	17 (6)*	17	14	0

*The number in parenthesis indicates the number of cases in which the wound had closed.
†One papilloma was noted in 1 case.

peared in large numbers. In those instances in which the graft contained mast cells (fig. 4 *C*), many of these had migrated into the adjoining wound base.

Of the 26 autotransplants of this type, 17 were found partly or wholly alive, while 9 had died, infiltrated by mononuclear and polymorphonuclear leukocytes (table 3). The living transplants showed little retraction and therefore were in closer contact with the marginal epithelium than the unpainted skin grafts. The effect of methylcholanthrene was indicated by thickening and increased keratinization of the epithelium of the transplants, with pegs reaching into the cutis. In addition, there were keratinized hair follicles as well as mast cells in varying numbers. Although considerable atrophy was present, mitoses were seen in the epithelium of the hair follicles (fig. 5). Here and there necrotic epithelium became replaced by cells migrating from living surface areas or from the depths of the living follicles. But whereas epithelial migration was seen within the transplant, the surface epithelium was not observed to grow out from the graft toward the marginal epithelium except in 1 case. Three specimens examined after seven or more days showed the transplant united with the wound base by an abundant

dense fibrous tissue. The latter contained epithelial cords with and without keratinization (fig. 6*A*). The surface of the transplant consisted of a discontinuous layer of hypertrophic stratified squamous epithelium. Dilated vessels were found in the cutis.

Series 4: Skin Painted with 20-Methylcholanthrene Was Transplanted into a Wound Made in Skin Likewise Painted with This Carcinogen.—The changes taking place under the influence of methylcholanthrene in the dermis and epidermis surrounding the wound were similar to those described in the painted skin of series 2. In 2 cases a papilloma was noted (table 4).

The regenerating epithelium of the wound margin was, from the beginning, closely approximated to the surface epithelium of the graft, since the apposition of these transplants was good. In spite of this close approximation, however, the regenerating epithelium showed no tendency to merge with the transplanted

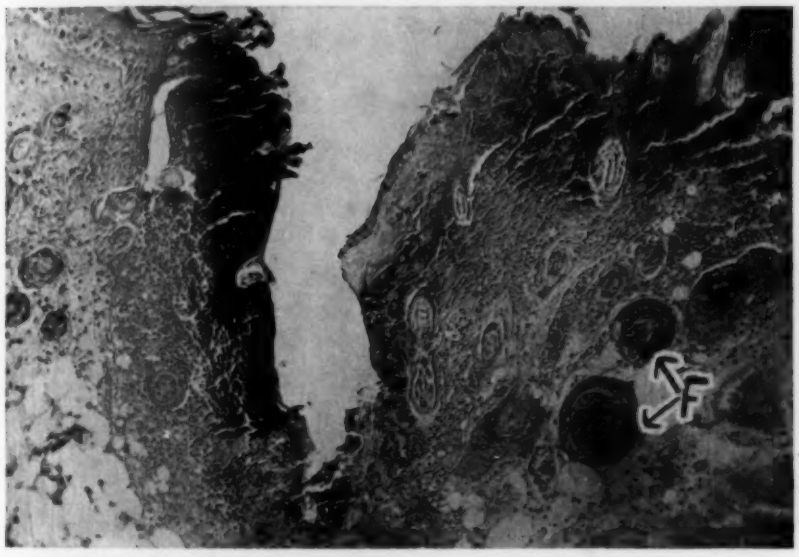

Fig. 5.—Autotransplant (painted skin grafted into wound made in untreated skin) two days after grafting; × 190. Two preserved hair follicles (*F*) may be seen in the transplant.

epithelium. The regenerating, heavily keratinized epithelium formed short, thick "tongues" composed of as many as six rows of cells (fig. 6*B*). Cellular infiltration of the wound base was slight. After five days the base consisted of a loose granulation tissue with some multinucleated giant cells. After seven days 1 of 8 wounds, and after ten days 5 of 8 wounds, had closed. In all 4 of the wounds examined, after fourteen days, repair had been completed.

Of the 52 autotransplants, 20 survived for periods ranging from one to seven days (table 4). They showed the aforementioned effects of methylcholanthrene in varying degrees. During the first three days, there was considerable atrophy, but mitotic proliferation went on for two or three days in the hair follicles, and here and there in focal areas of the surface epithelium. The epithelium of the hair follicles was seen to migrate somewhat toward the atrophic areas of the surface,

but subsequently cell movement declined. No outgrowing of the epithelium of the graft toward the margin of the wound occurred in spite of good apposition and close contact of graft and neighboring skin.

Series 5: Untreated Skin Was Transplanted into a Pocket Made Under Skin Painted Previously with 20-Methylcholanthrene.—One epidermal papilloma had appeared after two months, and one papilloma and one carcinoma, after three months of painting (table 5*A*). In the subcutaneous tissue, edema, vascularization,

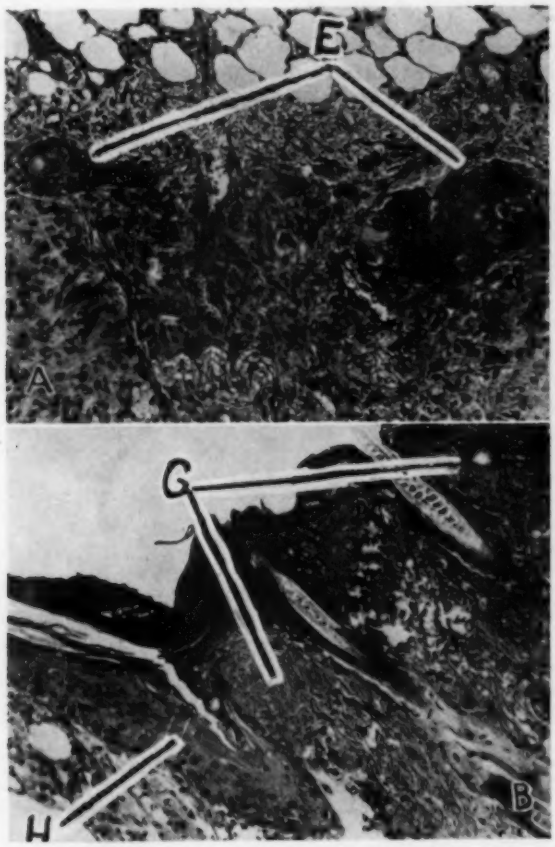

Fig. 6.—*A*, autotransplant (painted skin grafted into a wound made in untreated skin) fourteen days after grafting; × 90. Arrows indicate islands of transplanted proliferating squamous epithelium (*E*) in the granulation tissue of the wound base.

B, autotransplant (painted skin grafted into a wound made in painted skin) two days after grafting; × 9. Transplant (*G*) is in good apposition but shows no outgrowth. The marginal epithelium (*H*) of the wound is hyperplastic and shows a short thick "tongue" with little cell migration.

and fragmentation of collagen increased with the length of time during which methylcholanthrene had been applied to the surface epithelium.

The fate of the grafts depended on the intensity of the effect of methylcholanthrene on the overlying epidermis and corium. Of 36 grafts, only 9 survived.

TABLE 4.—*Autogenous Grafts: Skin Painted with 20-Methylcholanthrene Transplanted into a Wound Made in Skin Painted with 20-Methylcholanthrene*

Days After Transplantation	Grafts Made	Grafts Living	Main Observations		
			Proliferation of Epithelium of Graft, Cases	Migration of Epithelium in Graft, Cases	Outgrowth of Epithelium from Graft, Cases
1	8	5	5	4	0
2	8	3	3	3	0
3	8	4	4	3	0
5	8†	4	4	3	0
7	8†	4 (1)*	4	2	0
10	8	0 (5)*	0	0	0
14	4	0 (4)*	0	0	0
Total	52	20 (10)*	20	15	0

*The number in parenthesis indicates the number of cases in which the wound had closed.
†One papilloma was noted in 1 case.

TABLE 5.—*Autogenous Grafts: (A) Untreated Skin Transplanted into a Pocket Under Skin Painted with 20-Methylcholanthrene; (B) Skin Painted With 20-Methylcholanthrene Transplanted into a Pocket Under Untreated Skin*

Duration of Painting, Mo.	Days After Transplantation	(A)				(B)			
		Grafts Made	Grafts Living	Growth in Graft	Outgrowth and Cyst Formation, Cases	Grafts Made	Grafts Living	Growth in Graft	Outgrowth and Cyst Formation, Cases
½	2	2	1	1		2	0	0	
	4	2	2	2	1	2	0	0	
	7	2	0	0		2	1	1	1
	10	2	0	0		2	1	1	1
1	2	2	0	0		2	2	2	
	4	2	2	2	2	2	1	1	
	7	2	0	0		2	0	0	
	10	2	1	1		2	2	2	1
	16	2	0	0		2	0	0	
2	2	2	2	2	1	2	0	0	
	4	2	0	0		2	0	0	
	7	2*	1	1	1‡	2	2	2	1
	10	2	0	0		2	1	1	1
3	2	2	0	0		2	1	1	
	4	2	0	0		2	1	1	1
	7	2*	0	0		2	2	2	1
	10	2†	0	0		2	1	1	1
	16	2	0	0		2	1	1	1‡
Total		36	9	9	5	36	16	16	9

*One papilloma was noted in the painted skin overlying the graft.
†One carcinoma was noted in the painted skin overlying the graft.
‡Cysts were completely closed.

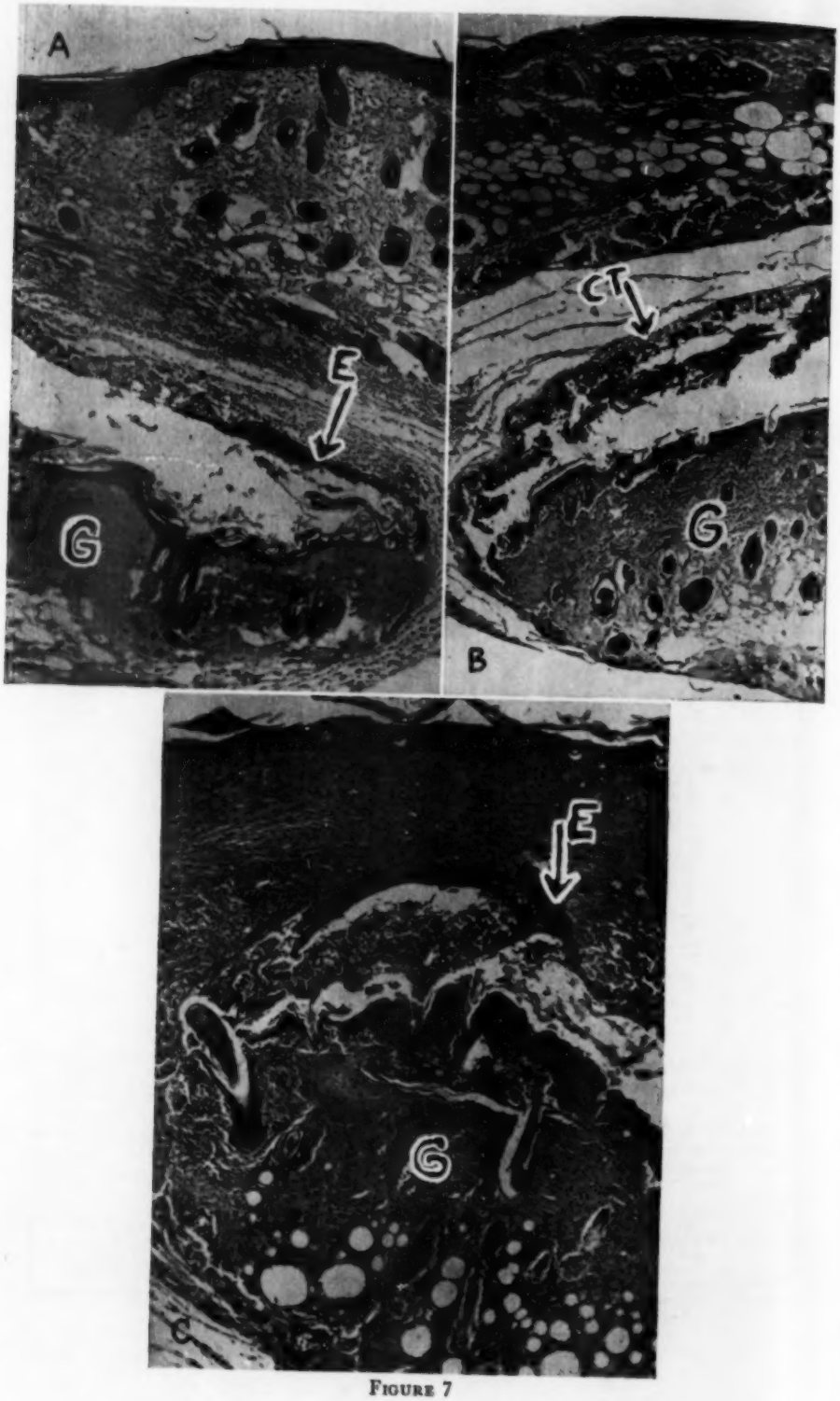

FIGURE 7

The more accentuated the effects of the carcinogen, the less favorable were conditions for the growth of the transplant, and the slower was the migration of the grafted epithelium. Of the grafts transplanted under skin painted for three months, none showed definite growth. Absence of growth was usually associated with marked edema and cellular infiltration around the graft. Not infrequently a granulation tissue with multinucleated giant cells replaced the dead transplant. The necrotic tissue was either resorbed or gradually pushed toward the surface. In the living grafts the basal cells and the epithelium of the hair follicles proliferated mitotically, and the former grew out in a circle or a semicircle. Migration of these cells was noticeable as early as one or two days after transplantation. Seven days after the grafting, the outgrowing epithelium began to form a cyst (fig. 7*A*). Cyst formation was the more complete, the less marked the cellular infiltration and edema of the subcutis.

Series 6: *Skin Painted with* 20-*Methylcholanthrene Was Transplanted into a Pocket Made Under Untreated Skin.*—Of 36 autotransplants of this type, 16 survived (table 5*B*). The more prolonged the application of methylcholanthrene, the more numerous were the instances of survival and the better was the growth of the epithelium of the graft. Mitotic proliferation of the surface epithelium and of the hair follicles were early signs of survival. Proliferation and migration of the elongated epithelial cells led to repair of necrotic foci within the transplant. However, compared with the markedly intensified mitotic activity of the epithelium the migration was less obvious: Outgrowing of epithelium from the transplant was not significant until seven days after grafting (fig. 7*B*), and cysts formed at a later date than in the less actively proliferating transplants of unpainted skin. Thus, whereas in the latter advanced cyst formation was observed seven days after transplantation, in the painted grafts a corresponding condition was noted only after sixteen days (fig. 7*C*). After that time an epidermal cyst consisting of several layers of epithelium and surrounded by a connective tissue capsule was found. The fate of the nonliving painted autotransplants was similar to that of the unpainted ones. They were either resorbed or extruded.

Homoiotransplants.

In the following series, 7 to 10, repair of the marginal epithelium followed a pattern comparable to that seen in series 1 to 4. However, there was in all wounds bearing homoiographs a delay of epithelization (tables 6 and 7).

Series 7: *Untreated Skin Was Transplanted into a Wound Made in Untreated Skin.*—The survival rate of the homoiotransplanted tissues was low. Five grafts showed growth during the first three days following transplantation (table 6). In 1 case some outgrowing of cells toward the margin of the wound was noted one day after grafting. All the transplants were cast off; they had undergone

Fig. 7.—*A*, subcutaneous autotransplant of untreated skin (*G*) grafted under skin that had been painted for one-half month with methylcholanthrene; it is seen here seven days after transplantation; × 65. Note that the outgrowing epithelium (*E*) is beginning to form a cyst.

B, subcutaneous autotransplant (*G*) of skin painted for one-half month previous to being grafted under untreated skin; × 65. There is no epithelial outgrowth. The free space above the transplant is due to retraction of the graft and desquamation of the keratinized surface epithelium. The free space is lined by connective tissue (*c t*) and contains desquamated keratin.

C, subcutaneous autotransplant (*G*) of skin painted for three months previous to being grafted under untreated skin; it is seen sixteen days after transplantation; × 65. Note outgrowth of the hyperplastic epithelium (*E*) with beginning cyst formation. The condition is comparable to that seen in *B*.

atrophy or been infiltrated by mononuclear and polymorphonuclear leukocytes. Five days after grafting, a granulation tissue had developed in the wound base, and here and there multinucleated giant cells took part in the destruction of the grafts. After seven days 1 of 4 wounds was closed, after ten days 3 of 4, and after fourteen days all 4 wounds were closed.

Series 8: Untreated Skin Was Transplanted into a Wound Made in Skin Previously Treated with 20-Methylcholanthrene.—Of the 28 homoiotransplants of

TABLE 6.—*Homoiogenous Grafts: Untreated Skin Transplanted Into a Wound Made in Untreated Skin*

Days After Transplantation	Grafts Made	Grafts Living	MAIN OBSERVATIONS		
			Proliferation of Epithelium of Graft, Cases	Migration of Epithelium in Graft, Cases	Outgrowth of Epithelium from Graft, Cases
1	4	3	3	3	1
2	4	1	1	1	0
3	4	1	1	0	0
5	4	0	0	0	0
7	4	0 (1)†	0	0	0
10	4	0 (3)*	0	0	0
14	4	0 (4)*	0	0	0
Total	28	5 (8)*	5	4	1

*The number in parenthesis indicates the number of cases in which the wound had closed.

TABLE 7.—*The Survival of Homoiogenous Grafts: (A) Untreated Skin Transplanted into a Wound Made in Skin Painted with 20-Methylcholanthrene; (B) Skin Painted with 20-Methylcholanthrene Transplanted into a Wound Made in Untreated Skin; (C) Skin Painted with 20-Methylcholanthrene Transplanted into a Wound Made in Skin Painted with 20-Methylcholanthrene*

Days After Transplantation	A		B		C	
	Grafts Made	Grafts Living	Grafts Made	Grafts Living	Grafts Made	Grafts Living
1	4	2	4	3	4	4
2	4	2	4	4	4	3
3	4	2	4	2	4	3
5	4	1	4	2	4	1
7	4	0	4	0 (1)*	4	0
10	4	0 (2)*	4	0 (2)*	4†	1
14	4	0 (4)*	4	0 (4)*	4	0 (3)*
Total	28	7 (6)*	28	11 (7)*	28	12 (3)*

*The number in parenthesis indicates the number of cases in which the wound had closed.
†Papilloma was present in 1 case.

this type, 7 were found alive during the first five days—2 after the first day, 2 after the second, 2 after the third and 1 on the fifth day (table 7*A*). Only little epithelial proliferation and migration were observed within the transplant, and these only during the first two days after grafting. The transplanted epithelium did not move toward the margin of the wound. All grafts underwent atrophy or

were infiltrated by mononuclear and polymorphonuclear leukocytes, and all were ultimately cast off. Edema, vascularization and cellular reaction of the wound base were more accentuated in the skin painted with the carcinogen than in untreated skin. Of 4 cases examined ten days after transplantation, the wound was closed in 2, and it was closed in all 4 examined in fourteen days.

Series 9: *Skin Painted with* 20-*Methylcholanthrene Was Transplanted into a Wound Made in Untreated Skin.*—Sixteen of these homoiotransplants were taken out during the first five days after grafting, and 11 of them were living (table 7*B*). Some proliferation and hypertrophy of the surface and follicular epithelium were noted, and there was some epithelial migration within the grafts. However, the epithelium of the transplant had not migrated toward the surrounding epidermis. Seven grafts were more closely attached to their wound bases, but the epithelial "tongues" advancing from the margins of the wounds undermined the transplants and separated them from the wound bases. After seven days all grafts had undergone regression and had been cast off. In 1 of 4 cases examined four days after transplantation the wound was closed; closure was completed in 2 of 4 cases examined after ten days, and in all 4 cases examined fourteen days after transplantation.

Series 10: *Skin Painted with* 20-*Methylcholanthrene Was Transplanted into a Wound Made in Skin Likewise Painted with This Carcinogen.*—Eleven grafts were found alive within the first five days following transplantation (table 7*C*). One additional graft, which carried a papilloma, showed, when ten days old, some proliferation and migration of the epithelium of the surface and the follicles. Ultimately all the grafts were destroyed by infiltrating mononuclear and polymorphonuclear leukocytes, or they were lifted off the wound base by the regenerating marginal epithelium. The wounds examined ten days after transplantation were still open. Of the 4 wounds examined fourteen days after transplantation, 3 were epithelized.

COMMENT

Autotransplantation.—In normal skin grafted into a defect of normal skin, the proliferating and outgrowing surface epithelium joined the epithelium growing out from the margin of the wound. This mechanism of direct union was observed in 68 per cent of the cases in which a graft of this type lived. Transplants thus incorporated in the wounds will be referred to as "primary takes." In normal skin grafted into a defect of painted skin, the surface epithelium showed some growth and migrated toward the margin of the defect. In 10 per cent of the cases the grafted epithelium underwent primary union with the marginal epithelium. In the remaining 90 per cent the transplant became atrophic and was cast off. Inflammatory changes in the wound base or undermining by the regenerating epithelium were responsible for the loss of the transplant. In painted skin grafted into a defect of normal skin, no outgrowing of the surface epithelium of the transplant toward the margin of the wound occurred; instead considerable regressive changes were noted. However, the surviving epithelium of the surface and the hair follicles proliferated and migrated over the denuded surface areas of the graft. At later stages the surviving epithelium of the transplant, in particular that of the hair

follicles, proliferated and grew into and over the granulation tissue of the wound. The results of the regeneration of these epithelial remnants migrating over the wound base may be designated as "secondary takes." They were found in 30 per cent of the cases in which this type of graft was made. In painted skin grafted into painted skin the epithelium showed no outgrowth and no tendency to unite with that of the wound margin. The graft ceased to grow and was finally cast off. In untreated skin transplanted under painted skin, early outgrowth and cyst formation were noted. Painted skin grafted under untreated skin showed survival and growth, and the more so the longer the period of painting prior to transplantation. However, the migration of the proliferating epithelium and the cyst formation were increasingly delayed.

TABLE 8.—*Comparison of Results*

	Autogenous Grafts				Homologenous Grafts			
	N/N*	N/P	P/N	P/P	N/N	N/P	P/N	P/P
Grafts made	52	26	26	52	28	28	28	28
Percentage of grafts living	65	54	65	38	18	25	39	44
Grafts examined 7 to 14 days after transplantation	20	10	10	20	12	12	12	12
Percentage of grafts alive 7 days after transplantation	75	25	75	50	0	0	0	0
Percentage of grafts alive 10 and 14 days after transplantation	67	0	50	0	0	0	0	12
Percentage of "primary takes"	68	10	0	0	0	0	0	0
Percentage of "secondary takes"	0	0	30	0	0	0	0	4

*The letters that head the columns indicate the experiments: They should be read as follows:
N/N—normal (untreated) to normal (untreated) skin
N/P—normal to painted skin
P/N—painted to normal skin
P/P—painted to painted skin

Homoiotransplantation.—Except for one graft that carried a papilloma, all homoiotransplants perished, although painted grafts persisted somewhat longer than unpainted ones.

Table 8 has been prepared to allow a comparison of the results observed in the various groups of transplants and to facilitate an evaluation of the findings. Each vertical column represents one of the experimental series 1 to 4 and 7 to 10. Horizontal column 1 gives the total number of grafts made in each group; column 2, the percentage of living grafts; column 3, the total number of grafts taken out seven to fourteen days after transplantation; column 4, the percentage of grafts alive seven days

after transplantation; column 5, the percentage of grafts living ten and fourteen days after transplantation; column 6, the percentage of "primary takes," and column 7, that of "secondary takes."

The over-all total of surviving autogenous grafts was the same (65 per cent) for normal and painted skin when these were transplanted into normal skin. "Primary takes" were noted in 68 per cent of the living grafts of normal skin made into normal skin. By contrast, none of the painted skin grafts showed "primary takes," but 30 per cent revealed "secondary takes."

Autogenous grafts, whether normal or painted, made in painted skin fared in all respects worse than the corresponding grafts made in normal skin. Altogether 54 per cent of normal and 38 per cent of painted transplants survived. Seven days after transplantation there was 1 "primary take" among 4 grafts of normal skin made in painted skin, bringing the total of "primary takes" in series 2 to 10 per cent. In painted grafts made in painted skin and examined during the second week, neither "primary takes" nor "secondary takes" occurred.

The percentage of surviving homoiografts was lower, and their time of survival shorter, than that of the corresponding autogenous grafts. In wounds made in normal skin 18 per cent of the untreated and 39 per cent of the painted grafts were alive during the period of observation, compared with 25 per cent of the untreated and 44 per cent of the painted grafts in wounds made in painted skin.

An "evenly balanced autogenous equilibrium" between the adjoining tissues[4] is a prerequisite for the "taking" of grafts. According to the work of Loeb,[4] autogenous equilibrium between adjoining tissues depends primarily on the identity of their individuality differentials, but there may be subsidiary factors. Thus, the fate of autogenous grafts under otherwise identical conditions depends on (1) the growth momentum of the transplanted tissue, (2) its resistance when subjected to injurious factors and (3) its ability to make suitable contact with the surrounding wound tissue and to avoid being dominated by the host tissue. This applies especially to grafts made in phylogenetically and ontogenetically more primitive organisms, but is probably true also of skin grafts made in mammals.

In the majority of autogenous grafts of normal skin based in normal skin the "autogenous equilibrium" was apparently present, since these transplants underwent primary union with the marginal epithelium. The failure of a number of these grafts to "take" must be attributed to technical imperfections, such as poor apposition or to some other incidental

4. Loeb, L.: The Biological Basis of Individuality, Springfield, Ill., Charles C Thomas, Publisher, 1945.

injuries, such as infection of the wound base and inadequate nutrition of the graft.

The decrease of the transplantability of normal skin grafts made into painted skin may be explained largely by changes in the wound base caused by the carcinogen. The edema of connective tissue and the fragmentation of collagen in the wound base make it difficult for the transplant to become firmly attached to the latter. The looser the contact between wound base and graft, the poorer the nutrition, and the more readily the transplant will be cast off. An additional unfavorable influence may be exerted by the regenerating epithelium of the margin of the wound. The painted epithelium of the wound margin has a far higher rate of growth than the graft. In spite of the relatively delayed migration of the painted epithelium, the latter will tend to epithelize the wound and to shut the graft off from its source of nutrition. Before undermining the graft, the marginal epithelium is apparently held up by the transplant and forced to make deep invaginations at its edge.

Notwithstanding their higher growth momentum, painted grafts did not establish contact with the regenerating unpainted wound epithelium. This might be due to the absence of outgrowth of the epithelium of the transplant. Neither did the normal skin regenerating from the margin of the wound grow over the painted grafts as it did over some unpainted grafts. Thus the carcinogen seems to have produced changes in the skin interfering with the establishment of a satisfactory autogenous equilibrium between the epithelium of the graft and the regenerating epithelium of the wound. Owing to this disturbance of equilibrium, no "primary takes" occurred. Some parts of the painted epithelium, however, survived the unfavorable conditions of the early stages of transplantation and regained their ability to grow and move over the wound with the establishment of better nutritional conditions. In these instances "secondary takes" were observed. Painted skin grafted into painted skin was in particularly good apposition, probably because both tissues were thickened and subject to less retraction than unpainted skin. However, the two apposed epithelial layers showed no tendency to unite. The poor growth and survival of grafts of this type were probably due to lack of epithelial outgrowth on the part of the graft and to edema of the wound base caused by the carcinogen.

The delayed migration and consequently retarded cyst formation seen in painted subcutaneous grafts corroborate our previous findings[1] on the healing of wounds in skin painted with carcinogens. Moreover, the significance of the state of the wound base for epithelial migration is illustrated by the fate of grafts of untreated skin transplanted under painted skin. The longer the carcinogen had been applied to the skin, the more pronounced was the subcutaneous edema. The more extensive the edema,

the less marked was the tendency of the grafted untreated epidermis to grow out and to form a cyst.

The application of methylcholanthrene did not alter the poor transplantability of homoiogenous grafts. These transplants were destroyed. However, in contrast to conditions in autogenous grafts, the carcinogen did not exert an additional harmful effect. Whether this observation is significant cannot be decided without further experimentation. The skin grafts carrying a papilloma and giving rise to a "secondary take" had apparently attained the growth momentum seen in neoplastic tissue.

Summarizing, then, we have not found it possible to increase the transplantability of the skin of mice by stimulating its growth momentum through the application of 20-methylcholanthrene as long as the proliferation has not attained the intensity found in neoplastic tissue. There is, on the contrary, a decrease in the transplantability of such epithelium as compared with untreated skin. It seems of interest to compare the present observations with recent findings by Rous,[5] who was able to activate skin grafts in rabbits by applying to the skin nonspecific irritants, such as turpentine, acetone and chloroform, previous to grafting. However, skin grafts may perhaps behave differently under the influence of nonspecific stimuli, on one hand, and of carcinogenic hydrocarbons, on the other. Similar differences were observed in the progress of wound healing[1] after application of benzene and carcinogen, respectively: Benzene accelerated wound healing, whereas carcinogenic stimuli delayed temporarily the progress of wound repair.

SUMMARY

20-methylcholanthrene applied to the epidermis of mice previous to transplantation intensifies the growth processes in the grafts taken from the skin thus treated. However, unless the transplanted epithelium has acquired neoplastic properties, the transplantability of painted autogenous grafts is decreased as compared with that of untreated skin. The poor transplantability of painted skin is due to factors present within the transplant as well as to conditions in the surrounding tissue. Such factors are the inhibition of the tendency of the epithelium of the graft to grow out toward the margin of the wound, failure of the marginal epithelium to make contact with the epithelium of the graft and the tendency of the marginal epithelium to undermine the transplant and to cast it off. Migration of the epithelium of the transplant may in particular be interfered with by edema of the surrounding subcutis as caused by the carcinogen. Surviving parts of painted grafts may continue to grow in the wound base and over the surface of the wound. The poor transplantability of homoiogenous grafts is not further decreased by the application of 20-methylcholanthrene.

5. Rous, P.: J. Exper. Med. **83**:383, 1946.

PULMONARY ALVEOLAR LINING IN BRONCHIECTASIS*

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THERE is no agreement regarding the normal microscopic anatomy of the respiratory portion of the lung. The bronchial tree of the fetus originally contains a complete lining of entodermal cells. Toward the end of the fifth fetal month, however, the epithelium of the terminal air passages begins to disappear and in the alveoli of the infant that has breathed for any length of time no traces of epithelium can be found.[1] Although under ordinary circumstances the alveoli of the adult do not have a visible lining of cells, a discernible lining of cells may be associated with many pathologic conditions of the lungs (table 1). This perplexing situation is largely responsible for the many discrepant accounts of the normal histologic structure of the pulmonary alveolus. It is our purpose to report the results of a detailed study of the pulmonary alveolus made in cases of bronchiectasis.

MATERIAL AND METHODS

Fifty human lungs that had been removed surgically because of bronchiectasis were taken indiscriminately from the pathology museum of the Mayo Clinic. In 4 of the 50 cases of bronchiectasis the lungs presented other pathologic processes as well (abscess in 2 cases, empyema in 1 case and tuberculosis in 1 case).

The lungs were examined carefully after having been fixed in solution of formaldehyde U.S.P. diluted 1:10. Tissue was chosen for microscopic study in the following manner: A block of tissue was removed so as to include one of the smaller grossly involved bronchi. A second piece of tissue was removed from the periphery of the most severely diseased portion of the lung. This block was cut so that it contained a portion of the visceral pleura. The cut surface of the lungs was examined for firm, gray, consolidated portions resembling those observed in the lungs of sheep suffering from jagziekte. From such portions tissue was removed whenever possible. The blocks of tissue were embedded in paraffin, and sections

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1. Barnard, W. G., and Day, T. D.: J. Path. & Bact. 45:67, 1937.

8 microns thick were cut and stained with hematoxylin and eosin. A few sections were stained for iron by the prussian blue method.

Pulmonary tissue and microscopic sections of tissues taken from animals suffering from jagziekte and chronic progressive pneumonia were obtained through the cooperation of Prof. E. V. Cowdry, of St. Louis, Dr. C. L. Davis, of Denver, and Dr. Hadleigh March, of Bozeman, Mont.

OBSERVATIONS IN THE FIFTY CASES OF BRONCHIECTASIS

Microscopic examination of the grossly consolidated portions of the lungs revealed that the interalveolar septums were markedly thickened. This thickening was due to an increase of fibrous tissue and to a cellular infiltration in which chiefly lymphocytes and plasma cells were concerned, with a few neutrophilic and

TABLE 1.—*Some of the Pathologic Conditions of Human Beings in Which an Alveolar Lining Has Been Described*

Bronchiectasis (1)	Infarcts (11)
Tuberculosis (2)	Chronic atelectasis (6)
Lipoid pneumonia (3)	Chronic fibrosis (6)
Interstitial pneumonia (4)	Pleurisy and empyema (12)
Bacterial pneumonia (5)	Lung abscess (6)
Viral pneumonia (6)	Gangrene (6)
Roentgen and radium pneumonitis (7)	Psittacosis (13)
Syphilis (8)	Epidemic influenza (14)
Irritation caused by war gases (9)	Pertussis (13)
Silicosis (6)	Toxoplasmosis (15)
Chronic passive congestion (10)	Bronchitis and bronchopneumonia (16)

1. Herbut.[6a] El Gazayerli.[7]
2. Simonds, J. P., and Curtis, J. S.: Arch Path. 19:287, 1935. Geever and others. [6c]
3. Herbut.[6a] Geever and others.[6c] Bell.[10]
4. Herbut.[6a] Bell.[10]
5. Sprunt, D. H., cited by Macklin, C. C.: J. Thoracic Surg. 6:82, 1936. El Gazayerli.[7] Geever and others.[6c] Klotz.[8] Miller.[9]
6. Geever and others.[6c]
7. Bauer, J. T., and Schraer, P. H.; Am. J. Path. 16:657, 1940. Warren, S., and Gates, O.: Arch. Path. 30:440, 1940. Geever and others.[6c]
8. Sprunt. Geever and others.[6c]
9. Groll, cited by Geever and others.[6c]
10. Parker, F., Jr., and Weiss, S.: Am. J. Path. 12:573, 1936. Sprunt. Geever and others.[6c] Bell.[10]
11. Simonds, J. P., and Curtis, J. S.: Arch. Path. 19:287, 1935. Geever and others.[6c] Sayre.[4]
12. Geever and others.[6c] Bell.[10]
13. Sprunt, D. H., cited by Macklin, C. C.: J. Thoracic Surg. 6:825, 1936.
14. Winternitz, M. C.; Wason, I. M., and McNamara, F. P.: The Pathology of Influenza, New Haven, Yale University Press, 1920, pp. 15-16. Sprunt (see no. 5)
15. Ash, J. E., and Spitz, S.: Pathology of Tropical Diseases: An Atlas, Philadelphia, W. B. Saunders Company, 1945, p. 197.
16. El Gazayerli.[7]

eosinophilic polymorphonuclear leukocytes. Numerous erythrocytes, mononuclear phagocytes and a few lymphocytes and polymorphonuclear leukocytes were present in the lumens of the alveoli. In 43 of the 50 cases of the alveolar walls frequently had a lining of rounded cuboidal cells. The nuclei of these cells were pale, contained a fine chromatin network and were usually round or oval. The cells contained abundant cytoplasm, which showed vacuolation and stained pale pink.

The arrangement of these lining cells varied. By far the most frequent finding was scattered, isolated plump cells or small patches of rounded cuboidal cells,

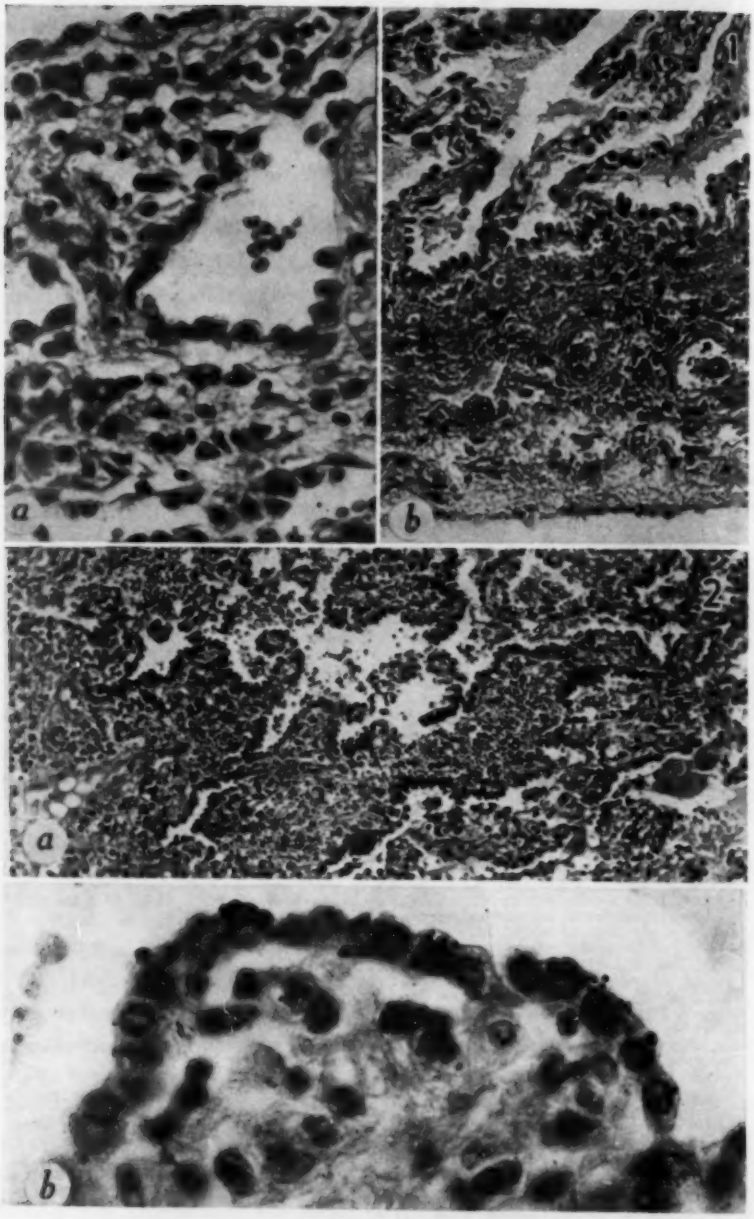

Fig. 1.—(*a*) Alveolus incompletely lined with isolated cells and small patches of cuboidal cells (hematoxylin and eosin; × 400). (*b*) Rounded cuboidal cells lining alveoli immediately beneath thickened pleura. The only portion of the alveolar wall that exhibits a lining of cells is the portion adjacent to the thickened pleura (hematoxylin and eosin; × 200).

Fig. 2.—(*a*) Alveoli lined with rounded cuboidal cells. The cytoplasm of the cells contains particles of carbon and the interalveolar septums are markedly thickened (hematoxylin and eosin; × 120). (*b*) Rounded alveolar cells forming a continuous alveolar lining. Particles of carbon are present in the cytoplasm of the cells (hematoxylin and eosin; × 740).

which lined the alveoli incompletely (fig. 1*a*). The alveoli bordering thickened pleura, heavy fibrous septums, areas of dense scar tissue, blood vessels or bronchi were more likely to be lined than those at other sites, and they usually contained larger patches of lining cells. Typically, only that portion of the alveolar wall abutting the fibrous tissue exhibited a lining of cuboidal cells (fig. 1*b*). Occasionally the alveoli were completely lined and in 6 cases there were regions in which practically every alveolus contained a complete continuous lining of cuboidal cells.

In 14 of the 50 cases the cytoplasm of the lining cells contained particles of carbon. Some of the cells contained only one or two particles of foreign material, while others were intensely phagocytic (fig. 2*a* and *b*). It was interesting that the lining cells in the alveoli bordering thickened pleura, heavy fibrous septums and areas of scar tissue were most frequently observed to be phagocytic. However, evidence of phagocytosis was encountered in all of the different arrangements of lining cells thus far described.

The pulmonary regions containing cell-lined aveoli were scrutinized for evidence that the epithelial cells grew down from the smaller bronchi, but none was found.

TABLE 2.—*A Comparison of Bronchiectasis and Jagziekte*

Bronchiectasis	Jagziekte
The Lining Cells	
Rounded and cuboidal	Square cuboidal and columnar, more frequently columnar
Cytoplasm vacuolated	Cytoplasm much clearer
Single layer	Sometimes several layers
Papillary folds not seen	Papillary folds observed frequently
Frequently phagocytic	Rarely if ever phagocytic
Interalveolar Septums	
Always thickened	Less marked; sometimes not present

Sometimes regions were noted which superficially resembled those seen in jagziekte.[2] (The reader should compare figure 3*a* with figure 3*b*.) However, careful scrutiny revealed many dissimilarities (table 2). In fact, the lining formation observed in cases of bronchiectasis resembled that seen in cases of chronic progressive pneumonia of sheep[2b,d] more closely than it did that in cases of jagziekte.

In 17 cases a study of the peribronchial tissues revealed alveoli, irregular spaces with many branchlike extensions, and small tubular structures containing respectively a lining of cells (fig. 4*a*). These lining cells differed from those previously described in that they were not rounded cuboidal cells but were definitely square cuboidal or low columnar cells. The nuclei stained darker than the nuclei of the cells previously described, and they contained a coarser chromatin network. The cytoplasm of the lining cells was more acidophilic and less vacuolated (fig. 4*b*).

2. (*a*) Cowdry, E. V.: J. Exper. Med. **42**:323, 1925. (*b*) Dungal, N.: Proc. Roy. Soc. Med. **31**:497, 1938. (*c*) Cowdry, E. V.: J. Exper. Med. **42**:335, 1925. (*d*) Cowdry, E. V., and Marsh, H.: ibid. **45**:571, 1927.

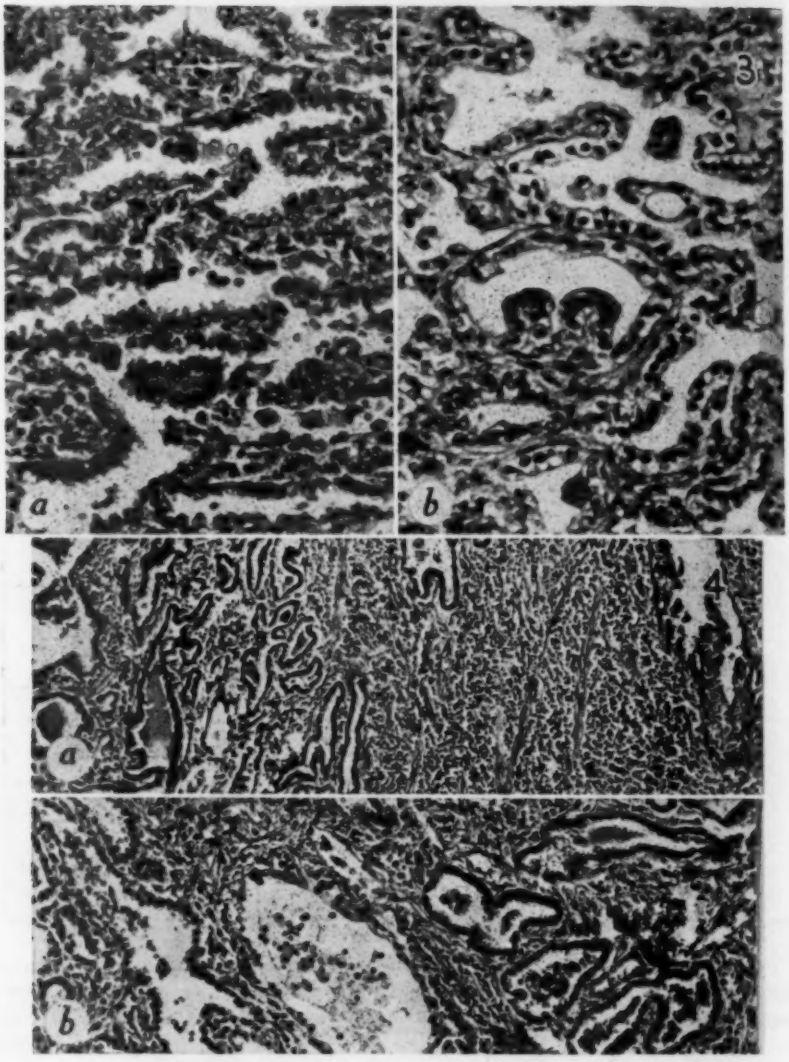

Fig. 3.—(*a*) Group of alveoli with a continuous lining of rounded cuboidal cells; thickening of interalveolar septums (hematoxylin and eosin; × 225). (*b*) South African jagziekte (section lent by E. V. Cowdry). This figure should be compared with figure 2*a* (hematoxylin and eosin; × 225).

Fig. 4—(*a*) Peribronchial tissue showing alveoli and irregular spaces lined with square cuboidal and low columnar cells (hematoxylin and eosin; × 80). (*b*) Peribronchial tissue showing the two types of cells lining the alveoli. The darkly stained square cuboidal cells on the right were presumed to be of bronchial origin. One can note the marked contrast between these cells and the lightly stained rounded cuboidal cells of the left (hematoxylin and eosin; × 115).

Ingested particles of carbon were never observed in the cytoplasm. These cells did not occur singly or in groups of two or three but occurred in larger patches which almost always completely lined the alveoli, the spaces or the small tubular structures. The tubular structures were often quite small; sometimes only four or five cells were required to complete their lining.

In 4 of the 17 cases bronchi were encountered in which downgrowths of the bronchial columnar and cuboidal cells could be traced into the peribronchial tis-

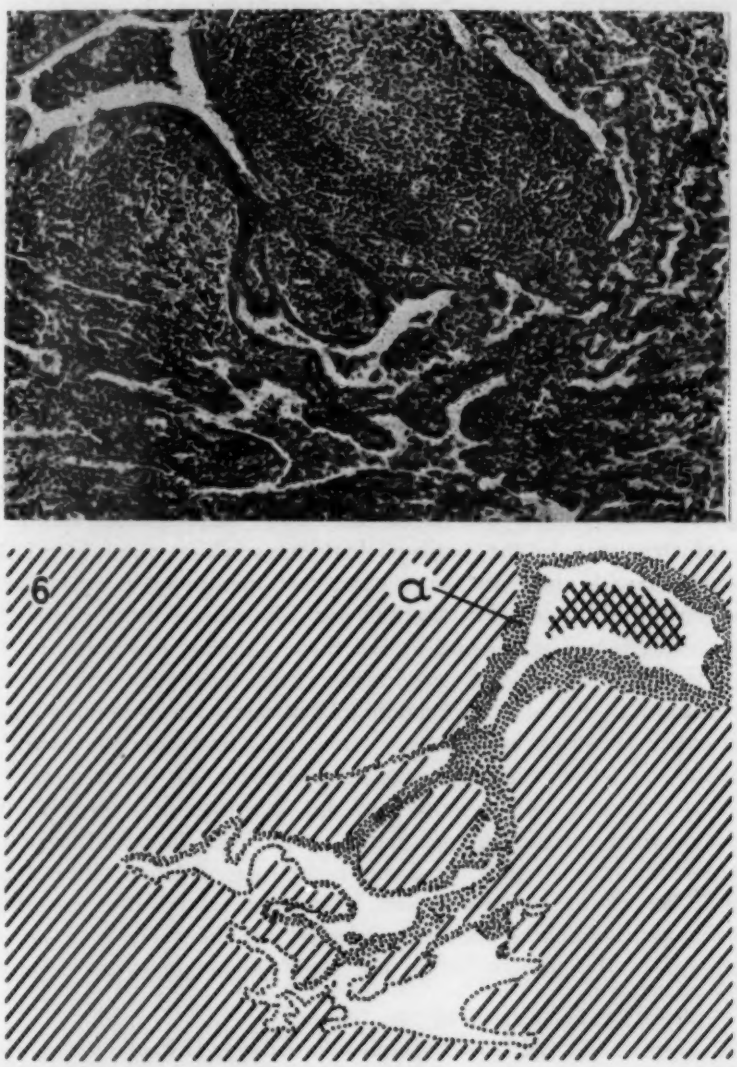

Fig. 5.—Epithelial cells growing down from a small bronchus. The cells have lined the adjacent alveoli (hematoxylin and eosin; × 85).

Fig. 6.—Schematic drawing of the section shown in figure 5. The epithelial cells actually can be traced from the small bronchus (*a*) into many of the surrounding alveoli.

sues where they formed a cubical lining for alveoli, small spaces and small tubular structures (figs. 5 and 6). If the bronchi were lined with the so-called transitional type of cells[3] or with squamous cells, the downgrowths at first consisted of transitional or squamous cells, but these soon assumed a cubical or low columnar shape. Examination revealed a few solid outgrowths of so-called pseudosquamatized cells and nests of hyperplastic squamatized epithelial cells growing independently of the bronchi which resembled those observed by Sayre[4] about pulmonary infarcts.

The findings in the 4 cases in which bronchiectasis was associated with other pathologic conditions (pulmonary abscess, tuberculosis and empyema) did not vary significantly from those in the 46 cases in which bronchiectasis was the only lesion.

These figures have been given only with the intention of indicating the relative frequency with which the various types of lining cells were encountered. The lungs were not examined by the serial section method. Moreover, the interpretation of many regions of pulmonary parenchyma was often precluded because of marked distortion of the tissues due to infections, collapse and fibrosis. It is obvious that at best statistical analysis would result only in rough estimates.

COMMENT

It was noted that in 4 of the 50 cases of bronchiectasis, bronchial epithelial cells actually could be traced from the smaller bronchi into the adjacent parenchyma, where they lined alveoli, irregular spaces and small tubular structures (figs. 5 and 6). These downgrowths of epithelium consisted of nonciliated square cuboidal or columnar cells with dark-staining nuclei and acidophilic cytoplasm. The cells were never observed to be phagocytic. In 13 cases there were alveoli, spaces and tubular structures in the peribronchial areas which were identical in appearance with those in the 4 cases just mentioned except that no downgrowth of bronchial epithelium could be seen without employment of serial sections (fig. 4*a* and *b*). In these 13 cases the lining cells resembled epithelial cells and were identical with those cells which actually were traced to the smaller bronchi. They were never observed to be phagocytic. It was presumed that these cells had likewise grown out from the smaller bronchi, although final proof of this was lacking.

It was evident from a review of the literature that bronchial epithelium, when sufficiently stimulated, will proliferate and sometimes grow out into the surrounding tissue in the form of small tubular structures or line alveoli and tissue clefts. This has been well demonstrated in experiments in which irritants were injected into the tracheas of animals.[5] In regard to the human being, the same phenomenon has been ob-

3. Fried, B. M.: Medicine **10**:373, 1931.

4. Sayre, G. P.: Epithelial Hyperplasia in Relation to Lung Infarcts, Thesis, University of Minnesota Graduate School, 1942.

5. Pinkerton, H.: Arch. Path. **5**:380, 1928. Willis, H. S., and Brutsaert, P.: Am. Rev. Tuberc. **17**:268, 1928. Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: J. Exper. Med. **32**:205, 1920. Fried.[3]

served in influenza[5a] as well as in many other bronchopulmonary diseases.[6]

It was noted that in 43 of the cases of bronchiectasis the alveoli adjacent to thickened pleura, thick fibrous septums and areas of scar tissue frequently were lined with cells which differed markedly from the cells which were proved or presumed to be of bronchial origin. The cells were rounded cuboidal cells instead of square cuboidal cells. Their nuclei were stained paler and contained a finer chromatin network than did the nuclei of cells of bronchial origin. Their cytoplasm was less vacuolated and less acidophilic. These cells were thought to be of alveolar origin for the following reasons: 1. They were frequently situated in alveoli abutting thickened pleura, fibrous septums or areas of scar tissue remote from the peribronchial regions (figs. 1*b*, 2, 3 and 4). 2. No evidence was found that epithelial cells grew down from the smaller bronchi. 3. The arrangement of the lining cells varied from scattered, isolated plump cells or small patches of cells incompletely lining the alveoli to a complete lining of cells (figs. 1*a* and *b* and 3*a*). This varied arrangement could easily be explained as the various stages of a process by which the lining developed from cells within the alveoli. 4. The appearance of the cells differed markedly from that of the lining cells of bronchial origin (fig. 4*b*). 5. The cytoplasm of these cells frequently contained particles of foreign material—chiefly carbon (fig. 2*a* and *b*).

The literature contains much evidence to support the concept of an alveolar origin of these lining cells. El Gazayerli,[7] Klotz,[8] Miller,[9] Bell,[10] Macklin[11] and others have expressed the opinion that the lining cells observed in the human being arose in the alveoli. Young[12] and El Gazayerli[7] held that the alveolar lining experimentally produced by the intrapleural injection of irritants arose in the alveoli. Cowdry[2c] studied serial sections of small lesions in cases of jagziekte and concluded that the lining cells often were not connected with the smaller bronchi. Spontaneous[13] and induced lung tumors[14] of mice also have

5. (*a*) Footnote 14, table 1.

6. (*a*) Herbut, P. A.: Am. J. Path. **20**:911, 1944; (*b*) Arch. Path. **41**:175, 1946. (*c*) Geever, E. F.; Neubuerger, K. T., and Davis, C. L.: Am. J. Path. **19**:913, 1943. Sayre.[4]

7. El Gazayerli, M.: J. Path. & Bact. **43**:357, 1936.

8. Klotz, O.: Canad. M.A.J. **17**:989, 1927.

9. Miller, W. S.: The Lung, Springfield, Ill., Charles C Thomas, Publisher, 1937, pp. 59-62.

10. Bell, E. T.: Am. J. Path. **19**:901, 1943.

11. Macklin, C.C.: J. Thoracic Surg. **7**:536, 1938.

12. Young, J. S.: J. Path. & Bact. **31**:265 and 705, 1928; **33**:363, 1930.

13. Slye, M.; Holmes, H. F., and Wells, H. G.: J. M. Research **30**:417, 1914. Wells, H. G.; Slye, M., and Holmes, H. F.: Cancer Research **1**:259, 1941.

14. Grady, H. G., and Stewart, H. L.: Am. J. Path. **16**:417, 1940.

been examined by the serial section method and have been shown to be of alveolar origin. Grady and Stewart[14] produced tumors in the lungs of mice by injecting 1,2,5,6-dibenzanthracene subcutaneously and observed that the growths typically developed in the subpleural alveoli. The first sign of the development of a tumor was some large mononuclear cells proliferating from the alveolar wall. These cells formed a partial or complete lining of the alveolar wall or coalesced to form small groups of cells. It was interesting that the cytoplasm of these cells occasionally contained particles of phagocytosed material. The authors wondered if the parent cell of the neoplastic alveolar lining might not be capable of differentiating into a cell indistinguishable from the alveolar phagocyte and then into an epithelial form. They cautioned, however, that this hypothesis could not be accepted without further study.

If it is true that the lining cells arose in the alveoli, it follows that they must have arisen from the pericapillary cells, since the remaining components of the alveolar wall are incapable of producing a lining. It was noted that in bronchiectasis the lining cells of alveolar origin often assumed both the arrangement and the structure of epithelial cells (fig. 3*a*); however, they were commonly observed to be phagocytic even when forming a continuous, complete histologic lining of cubical cells (fig. 2*b*). In addition, when not closely packed together they presented a distinct rounded appearance (fig. 1*a*). It was at once obvious that these cells displayed characteristics commonly attributed to both epithelial and mesothelial cells.

The fact that these cells were phagocytic did not prove that they were not epithelial cells, since foreign material has occasionally been observed in the cytoplasm of epithelial cells. Phagocytosis or a process resembling phagocytosis has been observed in tumor cells[15] and in the epithelial cells of the cornea,[16] the mammary gland,[17] the liver,[18] the male genital tract,[19] the vagina,[19] the bronchi[20] and the trachea and bronchi.[21] Ropes[21] found carbon particles in the ciliated epithelial cells

15. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 31. Dudgeon, L. S., and Barrett, N. R.: Brit. J. Surg. **22**:4, 1934. Behan, R. J.: Cancer, with Special Reference to Cancer of the Breast, St. Louis, C. V. Mosby Company, 1938, p. 126.

16. Ewing, J.: J. M. Research **12**:509, 1904.

17. Bratianu, S., and Guerriero, C., cited by Cowdry, E. V.: A Textbook of Histology: Functional Significance of Cells and Intercellular Substances, ed. 2, Philadelphia, Lea & Febiger, 1938, p. 365.

18. Tanaka, H., cited by Cowdry.[17]

19. Guieysse-Pellissier and Regaud and Tournade, cited by Duthie.[20]

20. Duthie, E. S.: J. Path. & Bact. **33**:547, 1930.

21. Ropes, N. W.: Phagocytic Activity and Morphological Variations of the Ciliated Epithelial Cells of the Trachea and Bronchi in Rabbits, Contrib. Embryol. **22**:77, 1930.

of the tracheas and bronchi of rabbits exposed to carbon dust. Sometimes carbon particles were found in the cytoplasm of small rounded ciliated cells that closely resembled (except for cilia, mononuclear phagocytes.

A study of lung tumors of mice revealed that an epithelium-like arrangement and structure of the cells did not prove that they were epithelial cells. In mice spontaneous[13] and induced[14] tumors arose in the subpleural alveoli and were typically epithelial in nature. Microscopically, the tumors resembled the lesions observed in jagziekte[22] (fig. 3*b*). When serially transplanted, however, these tumors were observed to change from typical epithelial growths to sarcoma-like lesions.[23] For this reason, many workers hesitated to classify the growths as epithelial tumors.

From the available evidence it was impossible to draw any conclusions regarding the origin of the pericapillary cells from which the lining cells of alveolar origin developed in bronchiectasis. However, the fact that the lining cells assumed characteristics commonly attributed to both mesenchymal and entodermal cells lent support to the hypothesis[24] that these cells and the alveolar phagocytes had a common ancestor. It is important to remember that this inference was drawn from incomplete evidence; the final solution must await further study.

SUMMARY

In bronchiectasis the pulmonary alveoli commonly had a visible continuous lining of cells. These cells were derived from two sources: first, from bronchial epithelial cells and, second, from the pericapillary cells of the alveolar wall. The latter origin was far more frequently encountered than was the former. The appearance of the cells of alveolar origin differed markedly from that of the cells of bronchial origin and, in addition, the cells of alveolar origin were frequently phagocytic even after they had assumed a typical epithelium-like structure and arrangement. Although no conclusions were drawn regarding the entodermal or mesenchymal nature of the lining cells of alveolar origin, it was conjectured that their parent cell also gave rise to the alveolar phagocyte.

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22. McDonald, S., Jr., and Woodhouse, D. L.: J. Path. & Bact. **54**:1, 1942.
23. Andervont, H. B.: Pub. Health Rep. **52**:347, 1937. Grady, H. G., and Stewart, H. L.: Am. J. Path. **15**:615, 1939.
24. Geever and others.[6c] Grady and Stewart.[14]

CONGENITAL MEGALOURETER AND HYDROURETER

Pathogenesis and Classification

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PEORIA, ILL.

FOR CONVENIENCE, we may divide congenital enlargement of the ureter into two groups: obstructive and nonobstructive. The first group includes enlargements based solely on a mechanical obstruction to flow of urine; the uterers may show congenital fibrosis, exaggerated constriction at points of anatomic narrowing, atresia, anomalous vesical insertion or valvelike folds. The second group includes enlargements without demonstrable obstruction; it may be divided into two subgroups. The first subgroup comprises nonobstructive enlargements associated with neurologic lesions as seen, for example, with spina bifida. The second subgroup comprises a relatively small number of ureteral enlargements for which no organic cause is found. It is with the last group of rather obscure cause that the present report deals.

Unexplained instances of ureteral enlargement have been known for many years. The attempts to classify them and clarify the genesis of the enlargement have resulted in much confusion in nomenclature. Anomalies which were formerly grouped under the heading "giant ureter" were later called megaloureter, after the introduction of this term, about twenty-five years ago. It was originally applied to a specific type of enlargement of the ureter but, as a result of its being used to designate many divergent forms of ureteral enlargement, its true meaning has become obscure. In the classification of ureteral anomalies seen in most textbooks of urology megaloureter is usually defined as a huge, dilated, atonic ureter without evidence of obstruction at its opening. In fact, a widely patulous or gaping orifice is characteristic. Hydronephrosis and atrophy of the kidney usually are accompanying conditions. As mentioned, megaloureter has been recognized in fetuses, newborn infants and children for many years, though accumulated evidence has shown it to be an uncommon cause of enlargement of the upper part of the urinary tract. Figures quoted by Campbell[1] reveal that it was encountered in only 7 of

From the Department of Pathology, St. Francis Hospital.

1. Campbell, M. F.: Pediatric Urology, New York, The Macmillan Company, 1937, vol. 1.

2,420 pediatric autopsies at Bellevue Hospital and in only 46 of 12,080 pediatric autopsies at four other hospitals—an incidence of about 1:275. In a review of 101 cases of hydronephrosis occurring in infancy and childhood Kretschmer[2] found that megaloureter was not observed as an associated condition in a single instance. Though usually bilateral, megaloureter may be unilateral. In the latter case, clinical symptoms may not appear, and the condition is discovered only at autopsy. If both ureters are involved, trouble usually develops early as a result of urinary stasis and subsequent infection. Saintu is credited with reporting for the first time the finding of giant ureters in a fetus. Following publication of his paper, in 1896, sporadic reports appeared during the next quarter century describing giant ureters, giant bladders and associated conditions.[3] The autopsy observations were never satisfactorily explained. Ureteral enlargement was diagnosed only at autopsy until urologic diagnostic procedures came into more widespread use. Caulk[4] did much to establish the condition as an entitity; in 1923 he reported a case in which the diagnosis had been made ante mortem by urologic study. He also introduced the term "megalo-ureter" to designate this type of nonobstructive congenital ureteral enlargement and likened it to Hirschsprung's disease in its genesis. Subsequent reports tended to substantiate such a relationship inasmuch as megaloureter was not infrequently found associated with other developmental anomalies, particularly anomalies of the digestive system.[5]

Admittedly, it is difficult to classify correctly the congenital ureteral enlargements. European investigators have studied the problem extensively, as is shown by the numerous reports in the foreign literature. These ureteral enlargements were usually designated as "giant ureters" before the term "megaloureter" was introduced. Ockerlad and Zinner and associates, [6] in reporting cases, emphasized the difficulty of distinguishing congenital from acquired megaloureter. Gaudino,[6] on the other hand pointed out that congenital megaloureter has an immovable vesical ostium in contrast to the acquired type. In the American literature little cognizance is taken of the thickness of the ureteral wall in identifying a particular type of enlargement. According to some authors, atonic,

2. Kretschmer, H. L.: Surg., Gynec. & Obst. **64**:634, 1937.

3. Maresch, O.: Wien. med. Wchnschr. **62**:249, 1912. Geraghty, J. T., and Frantz, W. A.: J. Urol. **2**:161, 1918. Papin and Legueu: Ann. d. mal. d. org. genito-urin. **22**:1361, 1904.

4. Caulk, J. R.: J. Urol. **9**:315, 1923.

5. (*a*) Braasch, W. F.: J.A.M.A. **73**:731, 1919. (*b*) Von Karuffa-Korbutt: Folia urol. **2**:167, 1908. (*c*) Etala, E.: Arch. argent. de enferm. d. ap. digest. y de la nutricion **19**:278, 1944.

6. Cited by Gruber, G. B.: Missbildungen des Menschen und Tiere, Jena, G. Fischer, 1913, vol. 3, chap. 3.

dilated ureters are encountered most frequently, whereas others find hypertrophy and dilatation most prevalent; both forms have been classed under the heading of megaloureter by some and under that of hydroureter by others, classifications serving to add to the confusion as to the true nature of the anomaly. Thus, in the present communication we shall review the prevailing concepts of the genesis of this poorly understood condition and, perhaps, by illustrating a simple classification with selected cases, clarify the problem at least in some measure.

PATHOGENESIS

When one attempts to discuss the genesis of congenital ureteral enlargements, it should be borne in mind that no single factor could account for the many diverse clinical and pathologic pictures seen. One gains the impression from the literature that when an explanation is accepted as being fairly plausible an attempt is made to account for most of the cases on this basis.

During the first part of the twentieth century it was believed that all enlargements of the urinary tract resulted from mechanical obstruction. But as more and more cases were studied, it gradually became evident that some other explanation must be sought in certain cases, in which the enlargement apparently was on a nonobstructive basis. Adherents of the concept of mechanical obstruction explained these cases by saying that a previously existing obstruction had disappeared by the time of examination. However, the nonobstructive enlargements present other features which help to identify them as a separate group. Therefore, the theory that there had been a previous obstruction could explain at best only cases of ureteral enlargement.

Gerard,[7] in extensive investigations, showed the ureteral bud to be wide and large up to the fifth month of fetal life in comparison with the rest of the urinary tract or the body as a whole. It is devoid of muscle until the eighth week, after which it grows and gradually envelops the ureter and the pelvis. With this information available, it should be easy to explain the various types of ureteral enlargements encountered. The thickened, hypertrophic type would represent a lack of the inhibition of ureteral growth that normally occurs at about the fifth month; thus, hyperplasia must result. On the other hand, one might assume that hypoplasia explains the thin-walled ureters, lacking in normal muscle development. Attesting to the validity of this embryologic theory, other developmental anomalies are not uncommonly found along with the enlarged ureter. We have already alluded to Caulk's original paper comparing megaloureter with Hirschsprung's disease. Braasch,[5a] Karuffa-

7. Gerard, L.: La forme de l'uretere chez le foetus et le nouveaune, Thesis, Paris, no. 63, 1908.

Korbutt[5b] and Rizzi[8] have reported this frequent combination. The available evidence strengthens the belief that one is dealing with a developmental malformation. The fact that these conditions are found even in fetuses lends more support to the concept of developmental malformation.

As pointed out in the introductory classification of ureteral enlargements, neurologic lesions may result in a condition simulating megaloureter in many respects. Therefore, it is important to search for a neurologic lesion before concluding that the condition represents a megaloureter of unknown cause. Furthermore, another concept of importance has evolved from investigations relative to the possibility that a neuromuscular disturbance plays a dominant role in the genesis of megaloureter. In discussing megaloureter occurring in sisters, Eisenstaedt[9] suggested nervous dysfunction as the etiologic mechanism involved in his cases. Later the concept was promulgated by Hurst,[10] who suggested that some types of megaloureter may result from a state of achalasia at the ureterovesical junction. Since then investigation has taken a new trend. In this connection we cite the work of Hepler,[11] a proponent of this theory which has gained widespread popularity and approval. Achalasia is based on a lack of coordination between ureteral peristalsis and relaxation of the ureterovesical sphincter. The sphincter does not relax when the impulse reaches it. This imbalance leads first to hypertrophy, then to dilatation of the ureters. The thickened, hypertonic, hypertrophic ureters represent a compensatory stage of ureteral function responding to this imbalance. However, this stage may be followed by one of broken compensation wherein the ureters become greatly thinned, atonic and dilated. Thus, this course of events may be initiated by a prolonged state of imbalance at the ureterovesical junction. Hepler also differentiated it from another type of dysfunction due only to spasm at the ureterovesical junction, which may interfere with urinary drainage. Superimposed on the ureteral changes subsequent to a prolonged state of achalasia are those of infection, so that the final result may resemble that of ordinary mechanical obstruction. And in cases of this type it is difficult at times to determine which was the primary disturbance. Much support for this argument has been gained by an attempt to regard all achalasias as having a common cause. It is now rather generally accepted that the dilation of the esophagus observed in cases of cardiospasm and that of the colon observed in cases of Hirschsprung's disease are due to achalasia of the cardiac and anal sphincters, respectively. We have

8. Rizzi, R.: Arch. ital. di urol. **12**:93, 1935.
9. Eisenstaedt, J. S.: Arch. Surg. **13**:64, 1926.
10. Hurst, A. F., and Jones, J. G.: Brit. J. Urol. **3**:43, 1931.
11. Hepler, A. B.: J.A.M.A. **109**:1602, 1937.

previously cited reports indicating that megacolon is found with megaloureter in numbers too great for these anomalies to be regarded as coincidental. More recently, from Brazil, Etzel[12] has reported his observations based on examination of 626 adults with various manifestations of megacolon, megaesophagus and megaloureter. Inasmuch as megaloureter is apparently common in certain sections of Brazil, a clinicopathologic study was carried out in an effort to determine its genesis and the role of contributing factors. Since it has been shown previously that the urinary and the digestive tract have similar intramural autonomic innervations, one would expect the two systems to react to disturbed function or pathologic change in the same manner. Etzel found degeneration of the intramural autonomic nervous system in the majority of his cases. This appeared to be a likely anatomic basis for the physiopathologic changes leading to the development of megacolon, megaesophagus and megaloureter. And in the cases which he reported, the common predisposiing factor was a prolonged deficiency of thiamine resultant from the poor dietary conditions prevailing in those sections of the country. Therefore, Etzel concluded that a degenerative change associated with dysfunction (achalasia) of the sphincter would appear to be the most likely cause of at least some types of magaloureter. However, it is difficult to apply this reasoning to the megacolon, megaesophagus and megaloureter seen in newborn and young infants. The finding of a rather rigid sphincter is not in accordance with the classic description of megaloureter. Futhermore, according to Bell,[13] the finding resembles more a paralysis of the ureterovesical sphincter permitting a reflux of the urine that is in the bladder.

Among other theories of less significance are included those having in view dilatation as a result of a chronic inflammatory process localized about the ureterovesical junction, spastic contraction of the bladder and ureteral stasis due to toxins. It is natural to assume that inflammatory processes involving the ureter should play a part in the development of megaloureter since inflammation is usually present at the time of examination. Braasch and Karuffa-Korbutt were among the early adherents of this theory. Whatever role inflammation plays, it must be secondary, as it is not seen constantly and never in the megaloureters found in fetuses. The other views are susceptible to the same criticism as the theory of inflammation and are included only for the sake of completeness.

With this introduction, illustrating the complexities of the situation, we shall present 4 cases with autopsies and comment on the genesis, the classification and the nomenclature of congenital ureteral enlargements.

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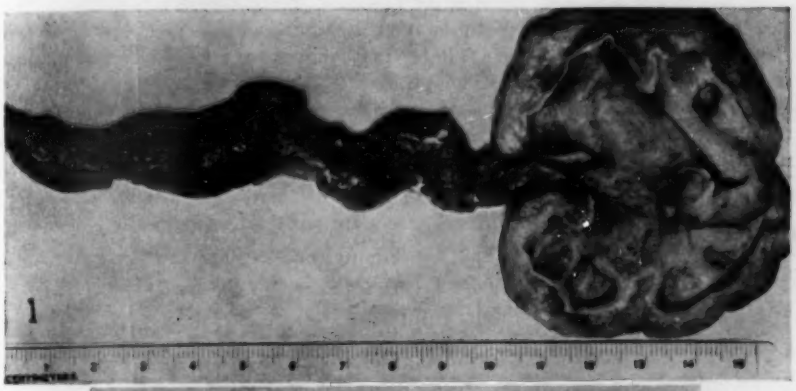
12. Etzel, E.: Am. J. M. Sc. 203:87, 1942.
13. Bell, E. T.: Renal Diseases, Philadelphia, Lea & Febiger, 1947, chap. 5.

REPORT OF CASES

CASE 1.—A 2 week old boy was admitted to St. Francis Hospital, in Peoria, March 14, 1947, because of jaundice, failure to gain weight, and enlargement of a mass that was felt in the upper part of the left side of the abdomen. The mass was soft, compressible, and displaced the intestine toward the right. The hemoglobin was 71 per cent; the urine contained a trace of albumin and showed 10 red and 20 white blood cells per high power field. Intravenous pyelograms failed to outline the kidneys. Surgical operation disclosed a "retroperitoneal



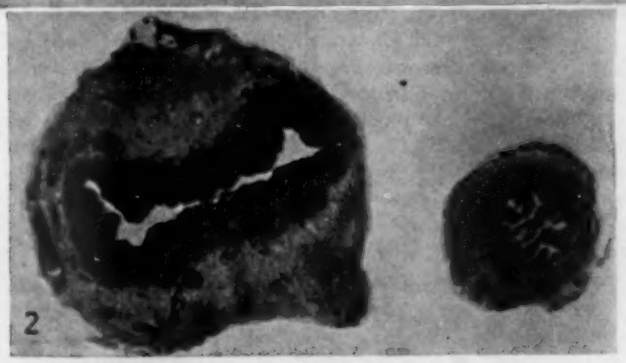


Fig. 1 (case 1).—Surgically removed left kidney and ureter. Note the marked thickening and tortuosity of the ureter as well as the marked hydronephrosis.

Fig. 2 (case 1).—Left: cross section of the ureter, showing great thickness of the wall and the narrowness of the lumen; × 5.5. Right: cross section of a normal adult ureter for comparison; × 5.5.

cyst" containing urine-like fluid. The cyst was attached to the left ureter. It was drained, and culture showed growth of Bacillus coli. The child's condition failed to improve; therefore, a second operation was carried out three weeks later, in which the entire kidney and ureter were removed (fig. 1). The ureter was thick and tortuous, but no obstruction was found. A previously occurring oliguria became more severe, and the course was progressively downhill to death, one week later.

Postmortem Examination.—The body was that of a 5 week old boy 52 cm. in length and weighing 2,850 Gm. The skin and the scleras were deeply icteric. The abdominal wall was thin and distended. The right kidney and ureter were similar to the left, which had been surgically removed. The pelvis and calices were dilated and filled with pus. The ureter was thick and tortuous but not noticeably dilated. There was no valve mechanism or obstruction at the vesical openings. The bladder measured 5.5 by 4.4 cm.; the wall was hypertrophic, measuring more than 1 cm. in width on the average. The urethra was patent throughout.

Microscopic Examination.—Cross sections of the ureter (fig. 2) showed thickening of all zones. Particularly thickened was the external zone of fibrous tissue and blood vessels; it was more than twice as thick as the muscularis and mucosa. The muscularis was well developed. Where it joined the external fibrous zone, lymphocytic infiltrations were abundant. The mucosa showed proliferated epithelium arranged in many layers. The subepithelial stroma was thickened and contained a moderate number of lymphocytes, plasma cells and eosinophils. The bladder wall was thickened as well, the increase being due mainly to proliferated fibrous tissue but also to muscular hyperplasia. The picture was similar to that of the ureter.

Diagnosis of Conditions Observed in the Urinary Tract.—Bilateral megalobladder; bilateral pyonephrosis and ascending purulent nephritis.

Case 2.—A 3 year old boy was admitted to St. Francis Hospital in June 1946 with a history of having had frequency of urination, dysuria and "strong urine" for one year. Albuminuria was discovered three months prior to hospitalization. When admitted he had a temperature of 102 F., taken rectally. He looked chronically ill, but the clinical findings were not remarkable. The red blood cell count was 3,300,000; the hemoglobin was 62 per cent; the urine contained albumin (2 plus) and showed 50 red and 10 white blood cells per high power field. The sediment contained many bacteria. The blood nonprotein nitrogen was 29 mg. per hundred cubic centimeters. Cystoscopic and roentgenologic studies showed widely patent ureterovesical orifices and severe hydronephrosis and hydroureter (fig. 3). There was no evident obstruction. Bilateral nephrostomy was carried out, with some improvement in the child's condition. In addition, multiple transfusions of blood were given as well as chemotherapy. The temperature was constantly elevated, ranging as high as 103 F., and the output of urine varied between 300 and 1,000 cc., with an average intake of 2,000 cc. In July the patient was discharged as slightly improved.

Six months later, in January 1947, he was readmitted because of chills, fever and diminution of urinary drainage following a mild infection of the upper respiratory tract that occurred two weeks previously. He was acutely ill. The skin and the mucous membranes were pale. The urine contained albumin, many erythrocytes and leukocytes, and bacteria. An abscess was found about the right nephrostomy tube. This was drained and the openings were enlarged. The child was discharged slightly improved, with his temperature curve still of a septic type, after twelve weeks.

One month after discharge the child was admitted moribund. He presented a shocklike terminal appearance. Death occurred twelve hours later.

Postmortem Examination.—The body was that of a 4 year old white boy 141 cm. in length, poorly nourished. The lungs were atelectatic in both lower lobes. The heart was enlarged (150 Gm.). The liver was enlarged and anemic, and showed gross fatty degeneration. Each kidney measured 8.5 by 4.5 cm. and showed a nephrostomy opening. The calices were dilated, the parenchyma being

reduced to 4 mm. in places. There were scattered cortical abscesses. The pelves and the ureters were dilated; the latter, to a circumference of 5 cm., and the wall was thickened to a width of 1.5 mm. There was no valve mechanism, abnormal insertion or other cause of obstruction at the ureterovesical junction. The bladder measured 6.5 by 7 cm. Its wall was thickened to a width of 1 cm. in places (fig. 4). The urethra was found to be patent throughout.

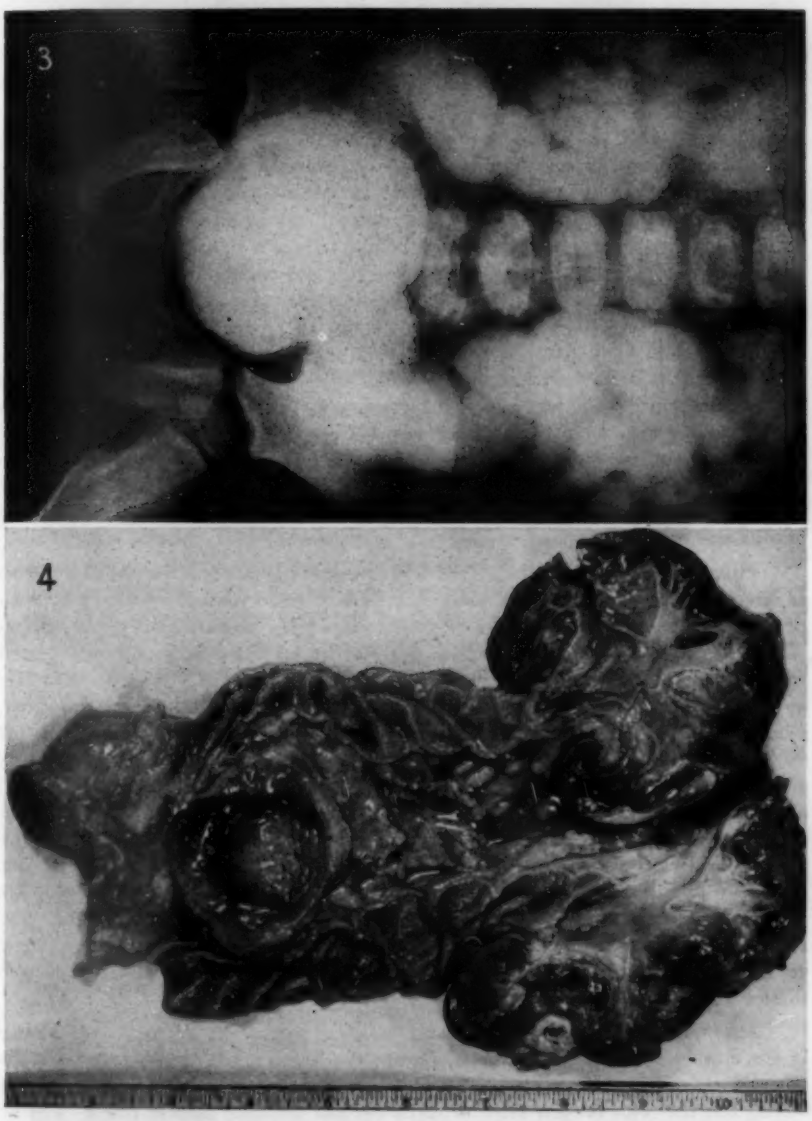


Fig. 3 (case 2).—Roentgenogram showing filling of the upper part of the urinary tract by a contrast medium injected into the bladder.

Fig. 4 (case 2).—Both ureters are markedly dilated and tortuous (megaloureter with secondary dilatation). Note also the hydronephrosis and the enlarged bladder.

Microscopic Examination.—Cross sections of the ureter showed thickening of the wall involving all zones. The mucosa showed proliferated epithelium with many lymphocytes and mononuclear wandering cells. The subepithelial stroma was well developed and showed lymphocytic infiltrations. Similarly, the muscularis was hypertrophic. The external fibrous zone showed proliferation to a lesser degree. The bladder wall presented the same picture in the mucosa and the submucosa. However, more than three quarters of the entire diameter of the wall consisted of hypertrophic muscularis. The external fibrous tissue was scanty.

Diagnosis of Conditions Observed in the Urinary Tract.—Megalobladder; bilateral megaloureter with dilatation; bilateral hydronephrosis with marked atrophy of the kidneys.

Case 3.—A 2 week old boy was admitted Sept. 24, 1947. At birth he appeared to be healthy, but the abdominal wall was lax and thin because of practically complete absence of musculature. In addition, a midline mass was palpable, extending from the umbilicus to the symphysis. This was thought to be the urinary bladder, but it never emptied completely. Previously studies made with barium sulfate had revealed no defect in the lower bowel. The child looked healthy, though urination was poor and feeding by stomach tube was necessitated. A catheter was passed with some difficulty and 29 cc. of urine obtained; culture showed B. coli. Cystograms revealed a large bladder with a superior projection. The upper part of the urinary tract was not filled with the barium sulfate. Since a congenital urethral stricture was suspected, a suprapubic cystotomy was carried out one week after admission. Thereafter an average daily output of 300 cc. was recorded with an intake of 500 cc. In spite of the improved urination, the infant failed to gain weight, and his general condition became steadily worse. Signs of bilateral basal pneumonia developed, and the child died three weeks after admission.

Postmortem Examination.—The body was that of a 5 week old boy 49 cm. in length. The adbdominal wall was thin, flabby and without musculature. A suprapubic catheter communicated with the bladder. About the catheter, in the wound, a quantity of greenish yellow pus was found. The lungs were edematous and had patchy pneumonic infiltrations, especially in both lower lobes. The gallbladder was in an abnormal position, and the entire colon had a free mesentery similar to that of the small intestine. The main observations pertained to the urinary tract. The urethra was narrow but patent throughout. The bladder was extremely enlarged, measuring when opened 10 by 8 cm. It was cylindric, with a cone-shaped fundus. The wall measured 5 to 8 mm. in thickness. The lumen was filled with pus, and the mucosa was coarsely granular, with multiple ecchymoses. Both ureter openings were patent but narrow. The left ureter was markedly dilated and tortuous, measuring as much as 2.8 cm. in diameter. The right ureter was somewhat less dilated. The wall measured as much as 1 mm. in thickness. Each kidney measured 5 by 3 cm. The parenchyma was reduced to a shell not more than 6 mm. in thickness. The pelvis and the calices were dilated (fig. 5).

Microscopic Examination.—Cross sections of the ureter showed little change in the epithelium. The subepithelial fibrous tissue was proliferated, forming a distinct layer of the ureter wall. The muscularis was largely replaced by fibrous tissue, but the Van Gieson stain brought out the atrophic muscle fibers. The external fibrous zone was thin and contained numerous blood vessels. Sections of the bladder wall showed the epithelium to be moderately thickened and arranged in many layers. The subepithelial stroma was thickened and contained several

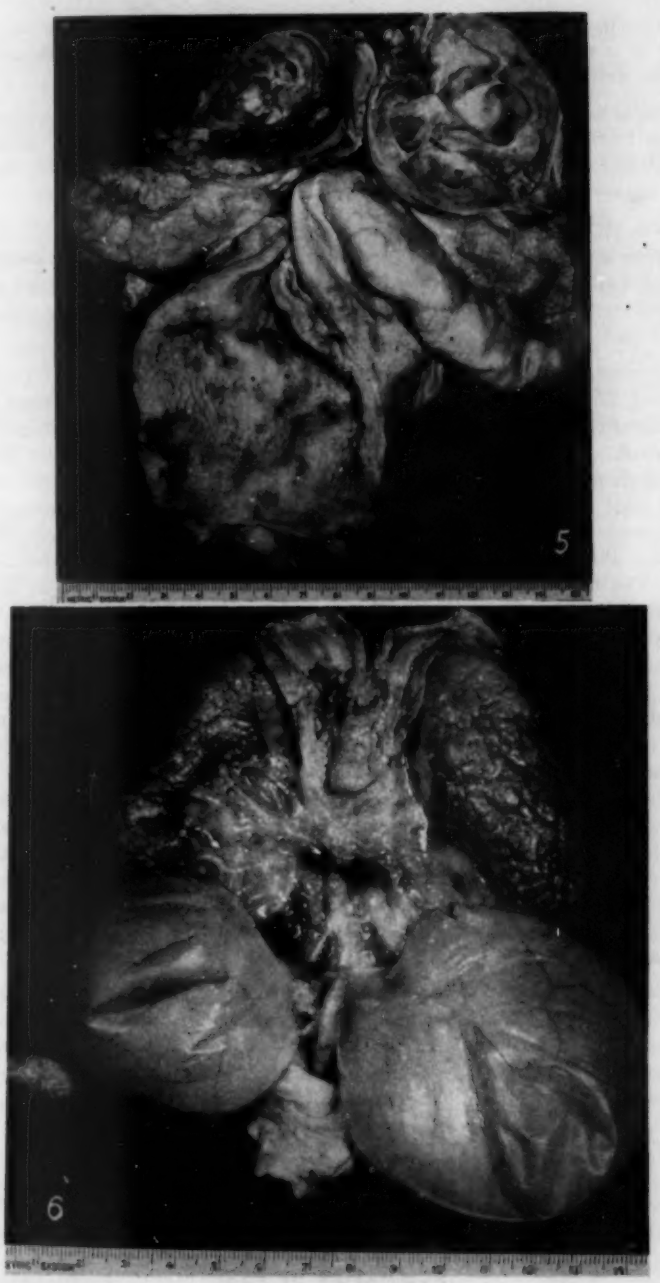


Fig. 5 (case 3).—Megalobladder, markedly dilated and tortuous ureters and hydronephrosis.

Fig. 6 (case 4).—Congenital bilateral hydronephrosis and hydroureter due to marked constriction of the ureteral orifices. In contrast with cases 1, 2 and 3, case 4 is not characterized by presence of tortuous ureters and megalobladder.

blood vessels. The muscularis was well developed. The muscle fibers were arranged in interlacing bundles, separated by fibrous tissue. The external fibrous zone was well developed and rich in blood vessels.

Diagnosis of Conditions Observed in the Urinary Tract.—Megalobladder with purulent cystitis and surgical fistula of the anterior wall; bilateral megaloureter with marked dilatation; bilateral hydronephrosis with hydronephrotic contraction of the kidney.

Case 4.—A premature girl was delivered spontaneously, Aug. 30, 1946, after seven and one-half months' gestation. The pregnancy was the mother's second and had progressed without incident until the premature onset of labor. After delivery, the infant lived only five minutes; it showed multiple developmental malformations.

Postmortem Examination.—The body was that of a white female infant measuring 37 cm. in length. There was generalized edema. The head was disproportionately large, with small, thickened ears. The face was flattened and the nose broad. The eyes were set widely apart. The neck was short and thick. The abdomen was proturberant, and there was a typical omphalocele, measuring 4 by 3 cm.; it contained several loops of small bowel. All four extremities were conspicuously short in relation to the trunk. The lungs were atelectatic. The liver was abnormally large, occupying more than one third of the abdominal cavity. The hepatic ducts were cystically dilated above a stricture at their junction, below which the common bile duct was not patent for a short distance. Bulging into the abdominal cavity were bilateral retroperitoneal cystic structures, which proved to be thin-walled, tremendously dilated ureters. Each kidney measured 7 by 4 by 3 cm. The surface was wrinkled, forming a pattern resembling convolutions of the brain. On section the parenchyma was reduced to a thin shell over a markedly dilated pelvis, measuring 4.5 by 4 cm. The ureteropelvic junction and adjacent segment of ureter on either side were relatively narrow, measuring 2.5 mm. in diameter on the right and 5 mm. on the left. Below these constrictions the ureters were converted into thin-walled cysts, the right measuring 5.5 by 5 cm. and the left 6.5 by 5.5 cm. When opened they were found to contain a clear fluid. The wall measured as much as 1 mm. in the thickest part. Beyond these cystic dilatations both ureters were stenosed. The bladder was small, measuring 1.5 by 2 cm. After the bladder was opened, the ureterovesical openings could not be probed (fig. 6).

Microscopic Examination.—On section the thickness of the ureteral wall varied from 0.5 to 1 mm. In the thicker portions thin bundles of smooth muscle could be identified, whereas in the thinner portions no musculature was found. Here the wall consisted only of fibrous tissue containing a moderate number of small blood vessels. The lining epithelium was cuboidal or flat and arranged in a single layer. No signs of inflammation were seen.

Diagnosis of Conditions Observed in the Urinary Tract.—Marked congenital stenosis of both ureteral orifices; marked bilateral hydroureter with hydronephrosis; hydronephrotic atrophy of the kidneys.

SUMMARIES OF CASES

Before discussing these 4 cases of congenitally enlarged ureters it may be helpful to summarize them briefly. The first case concerns an infant 5 weeks of age with a marked bilateral dilatation of the renal pelvis

and calices, enlargement and tortuosity of the ureters and enlargement of the bladder. The ureter wall was thick, while the lumen was not noticeably dilated. The bladder wall was likewise thickened. Histologic study showed the thickening of both ureter and bladder walls to be due mainly to fibrosis. No stenosis or other organic obstacle to the flow of urine was demonstrable along the entire urinary tract.

The second case was that of a child of 4 years with severe bilateral dilatation of the renal pelvis and calices, enlargement of both ureters, due mainly to dilation, and enlargement of the bladder. The ureter wall appeared to be disproportionately thickened, not commensurate with the marked dilatation present. The bladder wall was thickened, the width reaching 1 cm. Microscopic muscular hypertrophy was predominant, particularly in the bladder. Again no obstructive mechanism could be demonstrated in the urinary tract.

The third case was that of a 5 week old infant presenting hypoplasia of the musculature of the abdominal wall as well as malformations of the urinary tract. The latter revealed marked bilateral dilatation of the renal pelvis and calices, enlargement of both ureters due mainly to dilation, and tremendous enlargement of the bladder. The ureters were dilated, thin walled and tortuous. The bladder wall was thickened to a width of 1 cm. Histologic study showed the ureter wall to be made up chiefly of fibrous tissue, while the bladder wall contained well developed muscularis. Both ureterovesical orifices were narrow, though patent. Likewise the urethra was narrow but nonobstructing.

The fourth case was that of a premature infant with multiple congenital malformations. There was marked bilateral dilatation of the renal pelvis and calices, extreme enlargement of both ureters, due to dilation, and a small, contracted bladder. The ureters were stenosed at both the ureteropelvic and the ureterovesical orifices; the midportions were converted into thin-walled cystlike sacs; tortuosity was noticeably absent. Histologically, the ureter wall was composed only of fibrous tissue.

COMMENT

These cases seem to furnish a suitable basis for a morphologic classification of instances in which enlargement of the ureters is congenital. Since there is some uncertainty in the nomenclature of this condition, it might be helpful to establish the criteria by which we define the terms "congenital megaloureter" and "congenital hydroureter." Our first case is particularly valuable because it is an instance of true megaloureter. By this expression we understand an excess formation of ureteral tissue due to a faulty ureteral anlage which resulted in congenital hyperplasia of all elements of the ureter wall, especially of the fibrous tissue. As a result of this hyperplasia, the width and the length of the ureter became greater, and, as a result of the increase of length, tortuosity re-

sults, which is quite characteristic of megaloureter. On the other hand, in this case, the uretral lumen was not noticeably enlarged. The huge elephantiasic ureters composed mainly of fibrous tissue were not efficient as far as proper elimination of urine is concerned. This sluggish function with concomitant stasis and pressure effects led to more or less marked hydronephrosis. There was no physical obstacle to the flow of urine. The bladder was a typical megalobladder. In our opinion the case represents an unequivocal instance of uncomplicated congenital megaloureter.

The fourth case illustrates a ureteral lesion entirely different in its nature and genesis. Here the tremendous enlargement of the ureters represents hydroureter, the cause of which is a marked congenital constriction of the ureteral orifices. Both ureters are converted into thin-walled sacs, the walls of which are made up only of fibrous tissue with an atrophic epithelial lining. The condition leads also to obstructive hydronephrosis, which was marked in our fourth case. This type of congenital enlargement of the ureters differs morphologically from the hydroureter seen in adults only by its tremendous size, a difference explained by the fact that in postnatal life the patient would surely succumb long before this degree of hydronephrosis and hydroureter could develop. There was nothing in our fourth case indicative of a hyperplastic component accounting for the large size of the ureters. Particularly lacking were both the characteristic tortuosity and the megalobladder found in cases of congenital hyperplasia.

Between these two cases presenting entirely different types of congenital enlargement of the ureters there are cases in which there is evidence that a megaloureter was dilated secondarily to produce a condition presenting elements of both megaloureter and hydroureter. At autopsy the observations will depend to a certain extent on the duration of the strain on the ureter; after a certain period a secondary dilation of the megaloureter is to be expected. As mentioned the function of a megaloureter is inadequate, the urine not being able to pass through the thickened fibrotic ureter with the usual speed and in normal amounts. Thus, the resulting retention of urine leads finally to dilation of the ureters and the renal pelvis. The second case reported illustrates these changes. The tortuous course of the ureters, the thickness of the walls of the dilated ureters (which oppeared to be out of proportion to the degree of dilation), the presence of a megalobladder and the absence of an obstructive mechanism, all serve to indicate that here there was primarily a congenital megaloureter, which became secondarily dilated. The third case is somewhat similar in that malformations of the urinary tract were combined with agenesis of the muscles of the abdominal wall. This is a well known combination, reported many times in the literature. In this instance the megalobladder and the tortuous course of the ureters indi-

cated that the markedly dilated ureters were megaloureters in the sense just explained and that these giant ureters became dilated through retention of urine. In these cases emphasis is placed on the considerable thickness of the ureter wall as compared with the degree of dilatation of the lumen because we believe it indicates congenital hyperplasia rather than the compensatory or work hypertrophy postulated by Hepler and others. As illustrated in our third case the wall of the ureter was made up chiefly of fibrous tissue, whereas work should result mainly in muscular hypertrophy. As a matter of fact, in adult ureters subjected to similar influences the degree of work hypertrophy is negligible. The type of hypertrophy of the ureter described by Hepler results from what is in effect a functional obstruction at the ureterovesical junction, and its genesis is thought to be analogous to that of similar enlargements of the esophagus and the colon. We do not deny that some such mechanism may be present in certain instances, but we can state with reasonable certainty that in our cases, at least, the most plausible explanation is that the basis lies in congenital hyperplasia due to a faulty anlarge. Adding support to this concept is the fact that megalobladder was present in all 3 of our cases, which were called instances of megaloureter with or without secondary dilatation. In particular, the first case proves that we are dealing with an excess formation of ureteral tissue and resultant functional insufficiency leading to marked hydronephrosis. Such cases we may explain by developmental disturbances without resorting to pathophysiologic mechanisms, which are extremely difficult to verify, especially since one depends almost wholly on the morphologic examination in determining their likely pathogenesis. In contrast with these 3 cases, our fourth case represents an example of pure hydronephrosis due to stenosis of the ureteral orifices; it was used only for purposes of comparison, the enlargement having a pathogenesis different from that of congenital megaloureter as we understand the latter.

SUMMARY

We recognize three morphologic types of the congenitally enlarged ureter: (1) a megaloureter, representing congenital hyperplasia, which is manifested in thickened walls and increased length with tortuosity, without evidence of organic obstruction—an anomaly which has its analog in other pars of the body; (2) a dilated ureter (hydroureter), the result of the presence of an organic obstacle obstructing the flow of urine as seen in the obstructions acquired from any number of organic conditions; (3) an intermediate type representing a megaloureter which, because it has been unable to maintain normal flow of urine, has become secondarily dilated through retention. We admit that there will be cases of congenital enlargement of the ureter which, despite thorough morphologic examination, will be difficult to classify in any sense mentioned.

ANOMALIES OF THE CORONARY ARTERIES

Report of Four Cases

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THE coronary arteries are subject to considerable anatomic variation. For the most part, the variations consist in irregularities of the peripheral distribution of the vessels, so that either the right or the left artery supplies a greater portion of the myocardium than is usual. In such hearts the basic pattern of the stems of the left and right coronary arteries is usually maintained. The presence of a single coronary artery or an anomalous origin of one of the major branches, on the other hand, is sufficiently uncommon to be of great interest. For example, a recent review by Roberts and Loube[1] revealed that there were but 31 instances of single coronary artery reported, including the 9 of their own collection. There are isolated reports concerning anomalous origins of the left circumflex coronary artery.[2]

It was therefore of interest to us to discover 4 hearts in which there were anomalies of the major coronary arteries among 600 hearts which were being investigated primarily as part of another study. In that investigation the coronary arteries were examined in 100 unselected hearts of men in each of the six decades from the age of 30 through the age of 89 years. The coronary arteries were examined by means of transverse sections made every 3 mm. along the entire course of the major arteries and their grossly visible ramifications. It is the purpose of this report to describe the anatomic variations of the major coronary arteries which were found in these 4 hearts.

REPORT OF CASES

CASE 1.—The heart was obtained from a 39 year old man who had died as a result of cerebral glioma. There was but one coronary artery arising from the aorta. Its ostium lay in the right aortic sinus (fig. 1). There was no other coronary

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From the section on Pathologic Anatomy, Mayo Clinic.

1. Roberts, J. T., and Loube, S. D.: Am. Heart J. 34:188, 1947.
2. Antopol, W., and Kugel, M. A.: Am. Heart J. 8:802, 1933.

ostium. At its origin the single coronary artery showed a wide lumen, measuring 7 mm. in diameter. Four millimeters from its origin the artery divided into two branches. One encircled the posterior wall of the aorta, entered the left atrioventricular sulcus and then followed the course of a normal left circumflex coronary artery. The other branch of the single artery followed the course of a normal right coronary artery. Six millimeters from the origin of the left circumflex artery the single (right) coronary artery gave off a branch which showed an interesting course. Shortly after its origin this branch ran between the aorta and the pulmonary artery. While in this position it plunged into the upper portion of the ventricular septum and after running parallel with the upper portion of the septum, emerged in the anterior longitudinal sulcus to assume the course of a normal anterior descending coronary artery. Before this vessel emerged from the ventricular septum it gave off a relatively large branch which remained in the septum, coursed inferiorly and terminated near the apex of the heart.

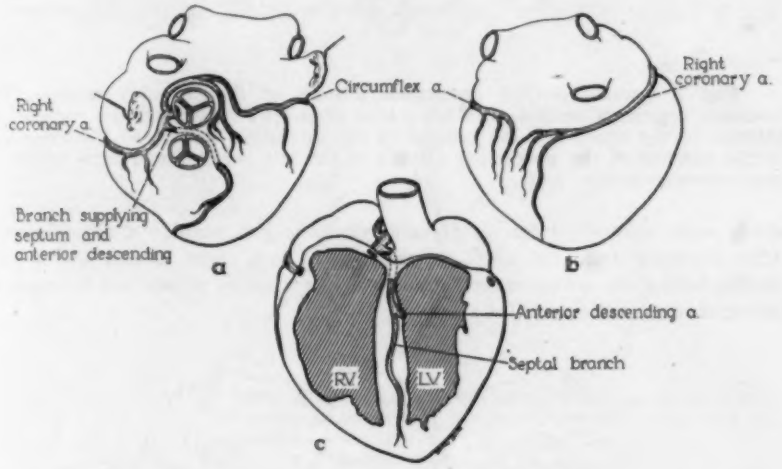

Fig. 1 (case 1).—The anomalous pattern of the coronary arteries. Only one coronary artery arises from the aorta. This is the right coronary artery from which the left circumflex and the anterior descending coronary artery arise in the order named. (*a*) Anterosuperior and (*b*) posterior view of the exterior of the heart. (*c*) The ventricles and the ventricular septum have been sectioned to show the course of the septal branch which arises from the proximal part of the anterior descending coronary artery.

Case 2.—The specimen was obtained from a man aged 77 years who had died of carcinoma of the sigmoid and cerebral infarction. The ostium of the right coronary artery was abnormally located, lying in the left aortic sinus immediately adjacent to the commissure between the right and left aortic leaflets (fig. 2).

Six millimeters to the left of the origin of the right coronary artery, the left coronary artery arose. The parietal (aortic) wall of the aortic sinus from which the two arteries arose was aneurysmally dilated. Microscopic examination of the involved portion of the aorta showed medial atrophy and fibrosis. This change was interpreted as a manifestation of acquired aortic disease, possibly syphilis. It did not, however, alter the concept that the origin of the right coronary artery was congenitally ectopic.

CASE 3.—The specimen in this case was obtained from an 81 year old man who had died of hypertensive cardiovascular disease with cardiac decompensation. This specimen was characterized by the anomalous origin of the left circumflex artery which arose from the right coronary artery (fig. 3). The right coronary

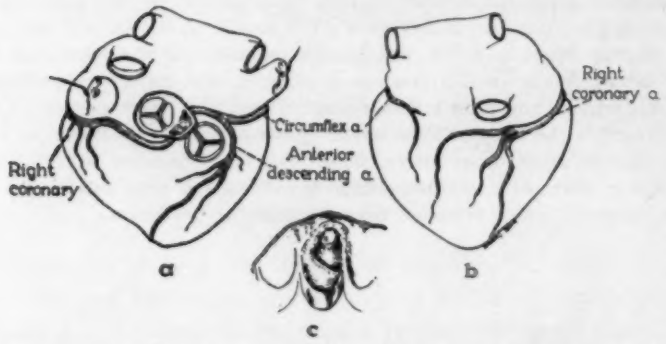

Fig. 2 (case 2).—The anomalous pattern of the coronary arteries. Both coronary arteries arise from the left aortic sinus. (*a*) Anterosuperior view of the exterior of the heart and the interior of the ascending aorta. (*b*) Posterior view of the exterior of the heart. (*c*) Details of the left aortic sinus, from which the two coronary arteries arise.

artery arose normally from the right aortic sinus and pursued a normal course. After emerging from the aorta it gave origin to a large branch which, after circling behind the aorta, entered the left atrioventricular groove and followed the course of a normal circumflex artery.

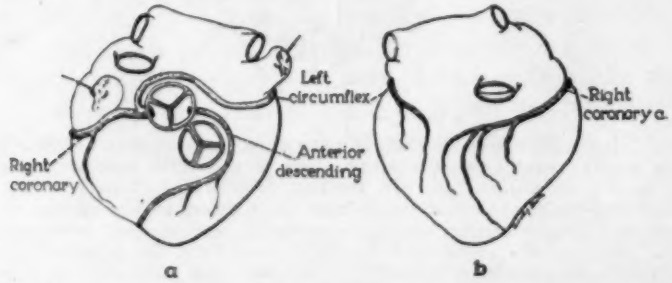

Fig. 3 (case 3).—(*a*) The anomalous pattern of the coronary arteries as seen from an anterosuperior view of the exterior of the heart and the interior of the aorta. The left circumflex coronary artery arises from the right coronary artery. The anterior descending coronary artery arises independently from the left aortic sinus. (*b*) Posterior view of the exterior of the heart.

As is normal, an artery arose from the left aortic sinus. This vessel which had an origin identical with that of a normal left coronary artery did not give off any major branches but merely continued into the anterior longitudinal sulcus as the anterior descending coronary artery.

CASE 4.—The specimen was obtained from a 76 year old man whose death was due to carcinoma of the sigmoid. The anomaly was similar in essentials to that of the specimen in case 3. The left circumflex coronary artery arose from

the right coronary artery (fig. 4). The only difference between the coronary artery patterns of the two specimens compared here lay in the fact that there was a somewhat more abundant distribution of peripheral branches of the circumflex artery over the posterior surface of the heart in case 4 than was evident in case 3.

COMMENT

Cases in which there is anomalous origin of the coronary arteries may be divided into two groups. In one group the anomaly consists in one or both coronary arteries taking origin from the pulmonary trunk. In the second group the coronary arterial system, though anomalous, is in communication with the aorta exclusively. The first group is serious since the heart is supplied with blood of low oxygen saturation. All the specimens described in this communication belong to the second group. The comments which follow will be concerned with the second group only.

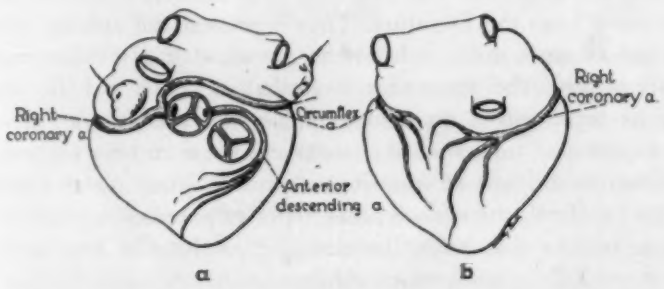

Fig. 4 (case 4).—The anomalous pattern of the coronary arteries is essentially like that in figure 3. (*a*) Anterosuperior view of the exterior of the heart and the interior of the aorta. (*b*) Posterior view.

Krumbhaar and Ehrich[3] and Roberts and Loube[1] have reviewed reported instances of single coronary artery in the light of the postulate of Hyrtl for the classification of true congenitally single coronary artery. According to these authors, Hyrtl stipulated that the criteria for true single coronary artery must include the finding that the entire heart is supplied by one coronary artery from which no conspicuous anomalous branches arise. By these criteria those cases in which the usual three major coronary arteries are present but in which there is but one coronary ostium in the aorta are not to be considered as examples of true single coronary artery, for the reason that the usual main branches have arisen from the single coronary artery stem on the basis of ectopia of their anlages.

Although there are instances which satisfy the rigid criteria of Hyrtl, there are others which, while not fulfilling the criteria of that author,

3. Krumbhaar, E. B., and Ehrich, W. E.: Am. J. M. Sc. **196**:407, 1938.

cannot be explained simply as examples of ectopia of anlages. Our first case affords an example of such an anomalous arrangement. It will be recalled that there was but one coronary ostium, which was in the postition of the ostium of a normal right coronary artery. The first branch of the single coronary artery was the left circumflex artery, which passed behind the aorta in coursing from its point of origin to the left atrioventricular sulcus. Had the anlage of the left circumflex artery been so ectopic as to make the artery arise from the right coronary artery, a position behind the aorta, which was present, would be anticipated. Similarly, the anterior descending artery would also be expected to pass behind the aorta if its anomalous origin had been caused by a misplaced anlage. But the course of the anterior descending artery was otherwise. Instead of passing behind the aorta, that artery coursed in front and to the right of the aorta, between the aorta and the pulmonary trunk. The second case of Krumbhaar and Ehrich[3] was essentially identical with our first case. The authors collected reports of similar cases from the literature. They expressed the opinion that while this group of cases did not fulfil the postulate of Hyrtl for true single coronary artery, the anomalies nevertheless could not be explained merely as representing an ectopic anlage of the anterior descending coronary artery. Under such circumstances these authors suggested that, in addition to the type of true single coronary artery which fulfilled the postulate of Hyrtl, there were other types of anomalous patterns of the coronary arteries that might be classified as those of true single coronary artery. This opinion seems valid to us.

In our second case the two coronary ostiums of the aorta lay in a single aortic sinus, the left. This case is of interest in that the anomaly may be interpreted as representing an intermediate stage between the normal, on one hand, and on the other, a condition wherein but one coronary artery arises from the aorta and from that artery, in turn, all the normal branches arise, the latter type of anomalous pattern resembling superficially that of the so-called single coronary artery but actually representing a misplaced anlage of the artery which is said to be absent. Such a case supports the concept that certain cases in which but one coronary stem exists do not represent true examples of single coronary artery from the developmental point of view.

It is interesting to speculate on the potential hazards to the person who has but a single coronary artery arising from the aorta. While anastomoses may be expected to occur between the various ramifications of that coronary artery as in those cases in which two major coronary arteries are present, the patient with but one coronary artery is at a disadvantage should occlusion occur in the single vessel proximal to the origin of any major branches. It is evident that should such an accident

occur the myocardium under ordinary circumstances would be deprived of most of its blood supply. That, in fact, such persons are more subject to myocardial infarction than are those in whom a coronary artery of normal pattern exists is suggested by the fact that of 28 patients living to adult life among 32 with single coronary artery,[1] 4 presented myocardial infarction. The expected incidence of myocardial infarction would be about 2 of 28 persons dying during adult life.[4] It is obvious, however, that while on hypothetic grounds the hazards of accidents occurring in the single coronary artery are expected to be greater, the data, while suggestive, are too few for one to draw hard and fast conclusions on this point.

Our third case was that of a man 81 years of age whose immediate cause of death was old and recent myocardial infarctions. Nevertheless, there seemed to be no casual relationship betwen the anomaly and the infarction.

SUMMARY

Three anomalous patterns of the coronary arteries are described. In each case, the aorta was the source of the coronary blood supply, and the anomaly was an incidental finding. All the patients were men.

In the first case there was a single coronary stem, which arose from the right aortic sinus. A short distance from its origin, this stem gave off the left circumflex artery. Immediately beyond, the anterior descending artery arose. This vessel, after entering the upper portion of the ventricular septum, gave off a septal branch and then proceeded in the normal direction of an anterior descending artery. The parent coronary artery, after giving off the branches named, continued as the right coronary artery.

In the second case both coronary ostiums were situated in the left aortic sinus.

In the third and fourth cases the left circumflex artery arose as a branch of the right coronary artery; the anterior descending artery arose independently from the left aortic sinus.

The developmental significance of anomalous coronary arteries is discussed. An occlusion occurring in a single coronary artery proximal to the origin of any major branches would cause the myocardium to be virtually without a source of blood.

4. Masters, A. M.: Am. Heart J. **33**:135, 1947.

PRODUCTION OF AN ANTIRETICULAR CYTOTOXIC SERUM

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IN CONSEQUENCE of the report of Bogomolets[1] that administration of the serologically standardized antireticular (antireticuloendothelial) cytotoxic serum made by Marchuk[2] may favorably affect carcinoma, we undertook the production of such a serum for the purpose of investigating its effects on the Brown-Pearce tumor of the rabbit.[3] This report is concerned with the preparation of the antireticular cytotoxic antigen, the immunization of animals resulting in the production of the specific antiserum, and the serologic titration of the serum antibody.

Normal tissue cytotoxins were first obtained by Dungern,[4] in 1899, by injecting ciliated tracheal epithelium of cattle into guinea pigs intraperitoneally. Observing the intraperitoneal fate of the epithelium, Dungern noted that cytotoxic changes occurred much more rapidly in the injected tracheal epithelium on subsequent injections, this tissue having shown relatively long viability on the first injection. Also in vitro studies in which tracheal epithelium suspended in normal guinea pig serum was contrasted with that suspended in "immune" serum obtained from guinea pigs into which identical tracheal epithelium had been previously injected demonstrated the cytotoxic effect of the antiserum. Shortly afterward Metchnikoff and Landsteiner independently were able to obtain serums toxic for spermatozoa.[5] Thereafter, at the turn of the century, in a period of about two years seventeen European reports, reviewed by Lambert,[5] were published concerning antiserums prepared for a total of ten different tissues. These tissues were leukocytes, liver, kidney, pan-

This work was supported by a grant from the S. Schiff Fund.

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1. Bogomolets, A. A.: Am. Rev. Soviet Med. 1:101, 1943.
2. Marchuk, P. D.: Am. Rev. Soviet Med. 1:113, 1943.
3. Saphir, O.; Movitz, D., and Strauss, A.: Cancer Research, to be published.
4. Dungern, V.: München. med. Wchnschr. 46:1228, 1899.
5. Lambert, R. A.: J. Exper. Med. 19:277, 1914.

creas, adrenal gland, thyroid gland, heart muscle, ovary, nerve tissues and syncytium.[5] Furthermore, it was reported that serum cytotoxins appeared to be species specific.[6] Regarding reticuloendothelial antiserums, ontogenetically related serums (antileukocytic) were studied by Metchnikoff[7] and several of his contemporaries. Later, Chew, Stephens and Lawrence[8] reported, in 1936, definite effects on administration of an anti-guinea-pig antileukocytic serum. Though splenic tissue or its extract has been used in experiments with malignant tumors for many years,[3] Marchuk's preparation of a combined spleen and marrow antiserum and Bogomolets' testing of it for its biologic effect on carcinoma represents the first attempt to use a tissue antiserum in such experimentation. Because of the heterophil antibody elicited on immunizing with guinea pig tissue, we shall describe the experience we have had with it in titrating guinea pig spleen and marrow antiserum. However, the antiserum studied for its capacity to affect the Brown-Pearce carcinoma was antirabbit,[3] and hence heterophil antibody was not involved in that study.

A serologically standardized normal tissue antiserum represented an advance over previously prepared normal tissue antiserums. As late as 1936[8] tissue antiserums were prepared and were studied without knowledge of their serologic titers. Marchuk[2] applied established serologic principles of the complement fixation test to a tissue antiserum. The tissue antiserum was obtained on injection of a rather crude antigen complex, which consisted essentially of ground normal red marrow and splenic tissue suspended in isotonic sodium chloride solution. The antigen used in the complement fixation tests was identical with that used for the immunization. Our preparation of the antigen, immunization procedures and titration of the antiserum are described in the following section of this paper.

METHODS

Preparation and Titration of the Antiserum.—We prepared the antireticular cytotoxic serum in accordance with the general methods used by Marchuk,[2] with some modifications. Because the serum was to be administered to rabbits for its possible effect and because of the apparent species specificity of such serum, according to previous investigators,[9] rabbit tissue antigen was utilized. A normal small mongrel dog was immunized by intravenous injections of freshly and aseptically prepared spleen and marrow antigen. The antigen was given in quantities increasing from 0.25 to 1 cc. at four or five day intervals until a satisfactory

6. Pomerat, C. M., and Anigstein, L.: Science **100**:456, 1944; Texas Rep. Biol. & Med. **3**:122, 1945. Bogomolets.[1] Lambert.[5] Strauss and others.[10]

7. Metchnikoff, E.: Ann. Inst. Pasteur **14**:369, 1900.

8. Chew, W. B.; Stephens, D. J., and Lawrence, J. S.: J. Immunol. **30**:301, 1936.

9. Bogomolets.[1] Dungern.[4] Lambert.[5] Chew and others.[8]

serum antibody titer was demonstrable. The dog was then bled peripherally for serum supply. The titer was determined by complement fixation.

Antigen.—The spleen and marrow antigen used for immunization or for complement fixation tests was obtained and prepared with aseptic precautions as follows:

A normal rabbit was rapidly exsanguinated as completely as possible by needle aspiration from its heart. Its spleen was then removed through an abdominal incision and placed in a mortar. The red marrow was blown out of, and collected from, each rib after total thoracectomy. The thoracectomy was accomplished by first exposing the thoracic cage both anteriorly and laterally and then completely opening it parasternally on one side. The vertebral column was then severed just above the thorax. The superior part of the thoracic cage was lifted upward (anteriorly) and its posterior aspect completely exposed down to the lumbar region. The peripheral attachment of the diaphragm was then cut, permitting the contents of the chest to be displaced inferiorly along with the liver, the stomach and the intestine. The lowermost thoracic vertebra, being exposed, was severed. The thoracic cage was then removed.

The sternum with the adjacent cartilaginous portions of the ribs was severed and discarded. On each side all of the ribs were removed in toto by cutting along a paravertebral line. Each rib was then freed from its attached intercostal muscle and fascia. The rib marrow was forced out, free of other "contaminating" tissue, when air was blown rapidly through the marrow cavity with a syringe and needle. This method was effective provided (1) the needle was inserted into the end of the rib's marrow cavity that had the smallest diameter, thus enabling free egress through the wider end, and (2) that each rib was divided into two at a point which eliminated the ineffectual forcing of air and marrow through a curved pathway. The marrow was delivered directly into the mortar already containing the spleen.

The weight of the marrow thus obtained from an exsanguinated rabbit, determined in a few instances, was approximately 0.4 Gm. The weight of the spleen was approximately 4 Gm. However, the relative proportions of spleen and marrow tissue varied somewhat with the animal and with the yield of marrow. This proportional variation apparently affected the anticomplementary titration of the antigen.

Spleen and marrow were then thoroughly ground in the mortar, and a total of 12 cc. of sterile isotonic sodium chloride solution was added gradually. This preparation was centrifuged for four minutes at 1,000 revolutions per minute. The thin top whitish fatty layer was pipetted off and discarded. The remaining supernatant was used on the day of its preparation as antigen for injection or for complement fixation titrations of serum antibody.

It was necessary that the antigen be used on the day of its preparation for complement fixation tests as well as for immunization. It was evidently unstable, deteriorating rapidly, and hence did not adequately demonstrate the presence of antibodies after that day. For example, an antigen that fixed complement with a specific antiserum diluted 1:80 on the day of its preparation fixed complement only in 1:10 dilution of identical serum when used twenty-four hours later. The antigen, prepared from the spleen and marrow of rabbits of approximately the same age and weight, was surprisingly uniform in the titrations. Four-tenths cubic centimeter of a 1:5 dilution of the antigen was usually the anticomplementary unit. It gradually became apparent that the greater the proportion of marrow in relation to spleen in the antigen used for titration, the greater was the tendency

for the antigen to be anticomplementary. One-tenth cubic centimeter of a 1:5 dilution of antigen, one fourth of the anticomplementary unit, was used for titration of antiserum.

Serum.—For titration the serum sample was inactivated by heating at 56 C. for thirty minutes.

Hemolytic System.—The antisheep rabbit system was used in the complement fixation tests. This system was titrated so that 2 units of antisheep amboceptor, 2 units of guinea pig complement and 0.1 cc. of a 5 per cent suspension of washed sheep red blood corpuscles resulted in complete hemolysis in fifteen minutes in a water bath at 37 C.

Complement Fixation Method.—The technic followed that of the original Wassermann reaction in one-tenth volume. Preliminary anticomplementary titrations of the antigen and of the serum and titration of the hemolytic system were made to determine the correct unit of each to use in the complement fixation tests.

Two series of complement fixation tests were made with each antiserum—varying the amount of antiserum in one instance and the amount of antigen in the other. In the first complement fixation test the antigen was constant: 0.1 cc. of a 1:5 dilution of antigen, representing one fourth of its anticomplementary unit, was added to each tube of a set containing serial dilutions of antiserum ranging from 1:10 to 1:320; each tube contained 0.1 cc. of the diluted antiserum. In the second test the serum remained constant: 0.02 cc. was used, and the antigen varied from one fourth to one four-hundredth of the anticomplementary unit (0.1 cc. of 1:5 to 0.01 cc. of 1:50). The mixtures of antigen, serum and complement were incubated for forty-five minutes in the water bath at 37 C.; then 0.1 cc. of a 5 per cent suspension of washed sheep red blood corpuscles and 2 units of antisheep amboceptor were added to each tube and the whole incubated at 37 C. for fifteen minutes. The customary controls—antigen, serum and hemolytic system—were set up with each test. The results of the complement fixation tests made by using these two methods have been recorded in tables 1 and 2, respectively. Subsequently, a third complement fixation test was included to demonstrate cold antibodies. The technic was similar to that employed in the tests recorded in table 1, but the inital incubation was 4 C. for overnight.

The results of the complement fixation tests made by the two methods, indicated in tables 1 and 2, are found, as is to be expected, somewhat similar. Four plus complement fixation occurred up to 1:80 with the serial serum dilution method and incubation at 37 C. (table 1). With serially diminishing quantities of antigen (0.2 cc. of a 1:5 dilution to 0.01 cc. of a 1:50 dilution of the antigen) being added to a constant amount of undiluted serum, 4 plus complement fixation was demonstrable up to 0.05 cc. of a 1:50 dilution of the antigen. In terms of absolute volumes of the serum and of the antigen preparation, the constant quantity in the instance of the antigen was 0.02 cc. (0.1 cc. of 1:5) and in the instance of the undiluted serum was 0.02 cc. Furthermore, 0.1 cc. of a 1:80 dilution of serum and 0.05 cc. of a 1:50 dilution of antigen represent approximately the same absolute quantity, 0.001 cc. The titer of the serum was found to remain stable for a period of about

four months, the serum being preserved in tightly stoppered tubes in the dark at 4 C. Its titer was checked at intervals.

A dog serum of higher titer was sought. Additional injections of antigen failed to stimulate production of antibody in higher titer. In fact, even decreasing titers were encountered. Two additional dogs, therefore, were subjected to a series of injections of antigen. These were given larger quantities of similarly prepared antigen, and the antibody production followed, as indicated in the chart. This chart indicates the rise in titer with both the interval of time and the additional antigen;

TABLE 1.—*Complement Fixation Titration of Antireticular Cytotoxic Serums: Serum Dilution Method*

							Serum Control	Antigen Control	Hemolytic System Control
Antiserum dilutions (0.1 cc.)	1:10	1:20	1:40	1:80	1:160	1:320	0.2(1:10)	0	0
Antigen (1:5 dilution*)	0.1	0.1	0.1	0.1	0.1	0.1	0	0.2	0
Complement (2 units)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		37 C. Water Bath for 45 Minutes							
0.1 cc. amboceptor (2 units) 0.1 cc. of 5% suspension of sheep red blood corpuscles	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		37 C. Water Bath for 15 Minutes							
Results (fixation)	4+	4+	4+	4+	±	0	0	0	0 (Complete hemolysis)

*The amount used was one fourth of the anticomplementary unit.

it also indicates the variations in titer of identical serum demonstrated by the different complement fixation methods. In addition to the serial serum dilution and serially diminishing antigen methods with initial incubation at 37 C., a third complement fixation method was included, similar to that employed in the tests recorded in table 1 but with the initial incubation at 4 C. overnight. This third method demonstrated cold antibodies in higher titer, 1:160.

TABLE 2.—*Complement Fixation Titration of Antireticular Cytotoxic Serum: Antigen Dilution Method*

	Antigen 1:5			Antigen 1:50					Serum Control	Antigen Control	Hemolytic System Control
Antigen	0.2*	0.1	0.05	0.2	0.1	0.05	0.02	0.01	0	0.2(1:5)	0
Antiserum (undiluted)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.04	0	0
Complement (2 units)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
			37 C. Water Bath for 45 Minutes								
0.1 cc. amboceptor (2 units) 0.1 cc. of 5% suspension of sheep red blood corpuscles	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
			37 C. Water Bath for 15 Minutes								
Results (fixation)	4+	4+	4+	4+	4+	4+	1+	0	0	0 (Complete hemolysis)	0

*The amount used was one half of the anticomplementary unit.

These studies indicate a limit to the production of antibody that may be elicited by a tissue antigen in a specific animal (the dog in this instance) when one is using rabbit spleen and marrow as antigen. Marchuk,[2] using human and rabbit tissue antigen, obtained high antibody levels in horses, sheep and goats in a relatively short time, complement being fixed in a dilution of serum of, for example, 1:1,280. Pomerat and Anigstein[6] and Strauss, Runjavac, Zaitlin, Duboff and Swerdlow[10] also elicited similarly high antibody production in goats with rabbit antigen. Miale[11] obtained high levels of antibodies for dog tissue antigen in rabbits.

The variation of the antibody titer demonstrated by the different complement fixation technics is indicated in the chart. The serum, demonstrated to be biologically potent by the effects observed when it was administered to rabbits with implanted Brown-Pearce carcinoma,[3] is shown to have yielded the highest detectable titer, 1:160 (0.0006 cc.), by the cold technic. The eventual titer of 0.05 cc. of 1:50 (0.001 cc.) was demonstrated by the diminishing antigen method, and the eventual titer of 1:80 (0.001) cc.) by the serial serum dilution method, both at 37 C. initial incubation. The differential rise of the demonstrable specific antibody titer of the serum, varying in accordance with the type of complement fixation as well as with the interval of time, is plotted in the chart. Titer determinations and their plotting according to absolute volume indicate the highest eventual titer rise to be demonstrated by the cold technic, and the next highest, by the diminishing antigen method, as found in 2 of the dogs.

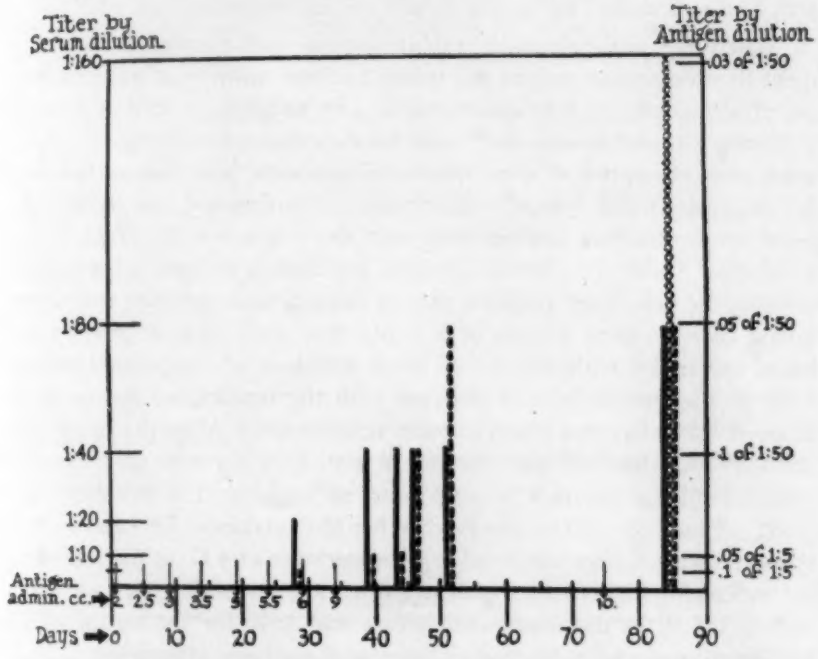
Antirabbit antireticular cytotoxic goat serum, obtained in the summer of 1947 through the courtesy of R. Strauss and co-workers,[10] was compared with our antirabbit antireticular cytotoxic dog serum. Goodner and Horsfall[12] have shown that antiserums elicited by identical antigen in different animals, such as mice and horses, present different properties, both serologically and biologically. Our titers compared closely with the titers obtained in Strauss's laboratories, but there was usually a decrease in titer by one tube, their 1:320 being 1:160 in our hands, and 1:160 being 1:80. This difference might in part be attributed to changes occurring during transit via airplane over a period of several hours, the serum vials usualy being packed in solid carbon dioxide at the start. In addition, the difference in titer may have been due to difference in the proportions of the two tissues composing the antigen used for the

10. Strauss, R.; Runjavac, M.; Zaitlin, R.; Duboff, G., and Swerdlow, H.: J. Immunol. 54:155, 1946.

11. Miale, J. B.: Blood 2:175, 1947.

12. Goodner, K., and Horshall, F. L.: J. Exper. Med. 66:413, 425 and 437, 1937.

immunization as well as in that used for the titration, and also to difference in technic. The results of our three complement fixation tests illustrate the difficulties encountered by investigators in different laboratories using different material in any attempt to obtain an identical numerical titer of a given immune serum. Normal goat serum, even undiluted, did not fix complement with this antigen. The serum of a normal dog showed an antireticular cytotoxic titer of 1:10. Serums of a second and a third normal dog yielded no complement fixation with the antigen prior to the administration of the antigen.



The progression of the titer of antireticular cytotoxic serum is determined by different complement fixation technics. The titer determined by using serial saline dilutions of the serum (incubation at 37 C.) is represented by an unbroken line; the titer determined by using serially diminishing quantities of the antigen, by a broken line; the titer determined by the cold technic (serial serum dilution), by a line of x's.

The rabbit hemolysin titer of the dog serum, determined before the serum was administered to the rabbits, was 1:16 on using 0.1 cc. of a 5 per cent suspension of washed rabbit red blood corpuscles, 0.1 cc. of amboceptor (dog serum) and 0.1 cc. of a 10 per cent solution of complement. The hemolysin titer of the goat antiserum against rabbit red blood corpuscles was also 1:16 by the same technic. Both normal dog and normal goat serums contained no detectable rabbit hemolysins.

We also undertook the production of antireticular cytotoxic serum for guinea pigs by injecting increasing quantities of guinea pig antigen into each of 3 rabbits at three to four day intervals. Exsanguination of the guinea pig and preparation of the antigen was done in the same manner as described for the rabbit antigen. For the first administration, one fourth of the total saline suspension extract of antigen of 1 guinea pig was injected intravenously into each rabbit. For the second administration of antigen, 2 guinea pigs were used; for the third and fourth administration of antigen 3 guinea pigs were used. Blood was drawn for titration on the seventh day following the fourth administration of antigen, and was drawn for serum supply on the following day.

When the anti-guinea-pig rabbit serums were titrated for complement fixation against guinea pig spleen and red marrow as antigen, prepared identically as for immunization, the titration yielded a titer of 1:320 for 1 rabbit's serum and 1:160 for the other 2 rabbits' serums, however, after absorption of their heterophil antibody. The technic followed for the antireticular cytotoxic rabbit serum titration was exactly like the serial serum dilution method used with the dog serum at 37 C. initial incubation (table 1). Because guinea pig tissues contain a heterophil antigen, the heterophil antibody titer of the unabsorbed serum was determined first by using 0.5 cc. of a 1 per cent suspension of sheep's red blood corpuscles with 0.5 cc. of serial dilutions of inactivated serum. Four plus agglutination was observed with the unabsorbed serum up to dilution 1:80 after two hours at room temperatures. After the heterophil antibody was absorbed from the serum with a 20 per cent suspension of guinea pig kidney extract for one hour, no agglutination of sheep's red blood corpuscles could be observed. When the mixtures of sheep erythrocytes and serums were subjected to a temperature of 4 C. in the refrigerator overnight, higher heterophil agglutination antibody titers were observed, 1:320 for the unabsorbed serum and 1:40 for the serum which had been previously subjected to heterophil antibody absorption.

To determine whether in complement fixation tests the anti-guinea pig rabbit serum causes hemolysis of sheep's red blood corpuscles, serial dilutions of the anti-guinea-pig serum were titrated for hemolysis of sheep's erythrocytes. The alpha hemolysin titer of the unabsorbed serum was 4 plus up to 1:80, with 1 plus hemolysis occurring as high as 1:1,280, after two hours at room temperature. The determination of the alpha hemolysin titer of the serum from which the heterophil antibody had been absorbed, showed only 3 plus hemolysis up to 1:20 and 1 plus hemolysis up to 1:320. When the same procedure was repeated without the addition of complement, the unabsorbed serum presented 4 plus beta hemolysis up to 1:40, and the absorbed serum showed no evidence whatever of the presence of beta hemolysins.

A comparison was made of the antireticular cytotoxic antibody titer before and after absorption of the heterophil antibody. Consistently, complement fixation occurred in much higher dilutions with the unabsorbed than with the absorbed serum. For example, a presumable antireticular cytotoxic antibody titer of 1:1,280 was observed with the unabsorbed serum, but the titer was 1:320 with the serum from which the heterophil antibody had been absorbed (table 3).

COMMENT

Three modifications of the complement fixation technic for detecting serum antibodies are demonstrated in the titrations of reticuloendothelial (spleen and red marrow) tissue antiserum. Somewhat different

TABLE 3.—*Complement Fixation Titration of Anti-Guinea-Pig Antireticular Cytotoxic Serum With and Without Absorption of the Heterophil Antibody*

	Complement Fixation in Given Serum Dilution							
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280
Unabsorbed serum plus guinea pig antireticular cytotoxic antigen	4+	4+	4+	4+	4+	4+	4+	4+
Serum Control (unabsorbed)	0	0	0	0	0	0	0	0
Absorbed serum plus guinea pig ARC antigen	4+	4+	4+	4+	4+	4+	2+	0
Serum control (absorbed)	3+	2+	0	0	0	0	0	0
		0.4 cc.		0.2 cc.	0.1 cc	0.05 cc.		
Antigen control (1:5 dilution)		±		0	0	0		
Hemolytic system		0 (no fixation)						

numerical titers of the identical serum were at times revealed by the three different methods. The first method consisted of incubating a series of saline dilutions of the serum in combination with a constant quantity of antigen (one fourth of the anticomplementary unit) at 37 C. The second consisted in using a constant undiluted quantity of immune serum in combination with serially diminishing quantities of antigen, also incubated initially at 37 C. Even though the serial serum dilution technic did not yield presumably adequate titers (at least 1:100), both it and the diminshing antigen method of titration gave positive results so far as a definite indication of the presence of the specific antibody was concerned. In view of the few animals immunized the serial serum dilution technic appeared to be the more sensitive during the early period of the immunization. However, when the antibody had reached higher levels,

the diminishing antigen method demonstrated the higher titer much more readily. Definite cytotoxic effects were being obtained experimentally with our preparation of 1:80 cytotoxic serum,[3] indicating that the serum was satisfactory from a biologic standpoint. The third method, the cold technic, demonstrated a titer well above 1:100. Thus, the cold technic and the diminishing antigen technic gave greater confirmatory serologic evidence that sufficient antibody or antibodies against a tissue complex were present in the dog antiserums.

With the use of such animals as the horse, the sheep, the goat and the rabbit, spleen and marrow immunization is reported to yield much higher levels of antibody after only a relatively small number of injections (four to six), and the confirmatory titers can be demonstrated by the usual serial serum dilutions.[13] This was proved to our own satisfaction when the rabbit was immunized with the guinea pig antigen.

The essential criterion as to whether a certain tissue antiserum contains the specific antibodies in question in sufficiently high titer, however, is the ascertaining of its intended biologic effect after it has been applied in vitro (nonserologic) or in vivo (by injection). In this instance it should be ascertained whether the serum has exerted the cytotoxic effect on tissue identical with that used in its preparation. This should occur regardless of the demonstrable antibody titer, and of necessity it was the first method used.[4] A certain degree of correlation, however, of the antibody titer and the biologic action of the serum may be expected. Serologic standardization is desirable, but that antibody titer should adhere strictly to certain specific limits is not a practical necessity. Bogomolets[1] stated that a titer of at least 1:100 is required of antireticular cytotoxic serum. However, in several experimental series in which the antiserum was found to be quite cytotoxic for reticuloendothelial tissue, the toxicity being evidenced by malignancy becoming enhanced with certain doses of the serum,[3] a serum titer of only 1:80 was demonstrated by the same general complement fixation method. During the course of the experiments, however, antiserum was produced yielding titers above 1:100.

Because heterophil antibodies are present in addition to the antireticuloendothelial tissue antibodies when such an antiserum is elicited with guinea pig tissue, conclusions drawn from studies made with such an antiserum cannot be unequivocably attributed to anti-retciuloendothelial tisue antibodies. Chew, Stephens and Lawrence,[8] studying the effect of an antileukocytic serum elicited by injecting guinea pig leukocytes into rabbits, recorded that the heterophil antibody titer of their antiserum was low. It is interesting to note serologically the presumably higher complement fixation titer of antireticular cytotoxic serum made

13. Marchuk.[2] Pomerat and Anigstein.[6] Strauss and others.[10] Miale.[11]

with guinea pig antigen when it was tested against similar guinea pig tissue, 1:1,280. However, the true antireticular cytotoxic antibody titer, 1:320, can apparently be known only by testing the serum after the heterophil antibody content has been absorbed. The antireticular cytotoxic antibody titer is then correspondingly much lower.

SUMMARY

A technic of preparing rabbit spleen and red bone marrow to be used as a tissue antigen (*a*) for intravenous administration and (*b*) for the complement fixation titration of the antiserum is presented. Immune serum was prepared by repeated intravenous injections of the antigen, which was given in increasing quantities. The titer of the immune serum was demonstrated by the complement fixation test, three modifications of which were tried. There may be slight variation in the numerical titer demonstrated by the three modifications. The cold method revealed the highest titer, and in most cases the serial antigen dilution method the next highest. At first, with the lower levels of antibodies, however, the serial serum dilution method appeared in our series the more sensitive. Antireticular cytotoxic serum was readily produced in 3 rabbits with guinea pig tissue antigen. However, a moderate amount of heterophil antibody was found which necessitated absorption of this antibody and retitration, a procedure which brought the antireticular cytotoxic antibody titer of 1:1,280 to 1:320. Antireticular cytotoxic serum is much more readily produced, and in much higher titer, in the rabbit than in the dog.

Case Reports

SUPERNUMERARY AORTIC CUSPS WITH MULTIPLE FENESTRATIONS AND WITH DISPLACEMENT OF THE LEFT CORONARY ORIFICE

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THE OCCURRENCE of supernumerary aortic cusps with multiple fenestrations and a left coronary orifice displaced above the aortic ring has not been previously reported.

These cardiac anomalies were incidental findings in a 47 year old white man who died of primary carcinoma of the bronchus with widespread metastases. These defects were of no functional significance but are of embryologic and developmental interest.

GROSS DESCRIPTION

There were four aortic cusps. The anatomic layout was that of a left and a right cusp, associated with a left and a right coronary orifice respectively, and two noncoronary cusps designated as the right posterior and the left posterior cusp. The right posterior cusp was small and appeared to have been inserted between the right coronary and the left posterior noncoronary cusp. The cusps were well developed and membranous, with a corpus arantii at the midpoint of each free edge. These cusps were so adapted to each other as to be competent to close the orifice. The sinuses of Valsalva were well formed except for large fenestrations in the commissural raphes which allowed the sinuses to communicate with each other. Single warty excrescences which measured 0.2 cm. in diameter, were situated on the ventricular surface of the right coronary and the left posterior cusp, about 0.2 cm. from the free edge. No thickening of the cusps or evidence of previous inflammatory disease was otherwise evident.

Multiple fenestras, most of which were of pinhead size, were present in the aortic and pulmonary semilunar valves. Twenty-three were found in the former; twelve, in the latter.

The mouth of the left coronary artery was displaced 1 cm. above the aortic ring. The course of this artery was otherwise not aberrant. The right coronary orifice was normally situated.

The heart was otherwise free of abnormalities. The only other anomaly encountered was fetal lobulation of the kidneys.

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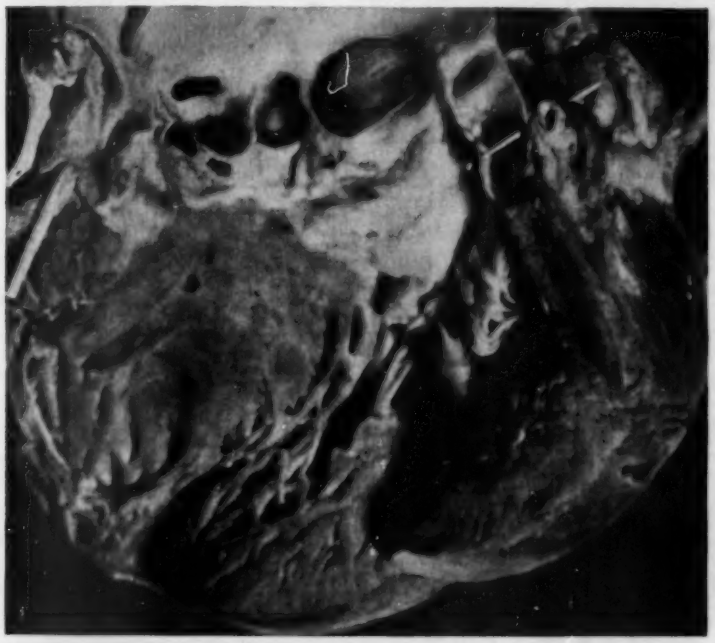
COMMENT

In the human embryo the aortic and the pulmonary valve are formed by division of the truncus arteriosus, the forepart of the early tubular heart. The dividing septum splits the four-cusped valve of the truncus arteriosus to form two valves, each with three cusps. Alterations of this symmetric arrangement occur from time to time, either valve being composed of two, four or even five cusps. Irregular growth of the aortic septum would account for cases in which there are only two cusps, one



Photograph of the heart (natural size) showing four aortic cusps with fenestrations. The left coronary cusp is divided, with a marking pin in each half. The coronary arteries are indicated with wooden rods.

or both lateral cushions remaining undivided. The fourth and fifth cusps appear to be developed later and are usually rudimentary. The explanation of the displaced coronary orifice is not known.

Supernumerary cusps may occur in the pulmonary artery and the aorta but are ten times more frequent in the former. Bicuspid aortic valve, in comparison, is not an uncommon finding, being observed in 0.56 per cent of autopsies, according to Gross,[1] who reviewed 5,000 consecutive records. The accompanying table reveals the number of cases of supernumerary aortic cusps reported in the literature.

1. Gross, L.: Arch. Path. **23**:350, 1937.

A more or less perfectly formed fourth cusp of varying size but usually smaller than normal may be inserted between two of the others, or the usual number of segments may exist and the sinus behind one of them be divided by a raphe which runs from the back of the cusps to the aorta, indicating that the additional segment has been fused with, or imperfectly divided from, its fellows. The supernumerary cusp has sometimes been explained as an effort to repair some inflammatory damage of long standing, but when the fourth segment is perfectly formed, as it was in this case, it must be considered a malformation. Further evidence in favor of a congenital origin is the association of other anomalies, the presence of thin, transparent valves, and the absence of postinflammatory manifestations.

Cases of Supernumerary Aortic Cusp Recorded in the Literature

Author	Cases of Supernumerary Cusp, or Cusps, Aortic Valve		Incidence
	4 Cusps	5 Cusps	
Dilg, J.; Virchows Arch. f. path. Anat. 91:193, 1883; cited by Wauchope	2	1	Total number of cases collected from literature
De Vries, W. M.: Beitr. z. path. Anat. u. z. allg. Path. 64:39, 1918	1	0	In 3,600 necropsies
Simonds, J. P.: Am. J. M. Sc. 166:584, 1923	2	0	In 15,666 necropsies, including the de Vries series
Wauchope, G. M.: Quart. J. Med. 21:383, 1928	0	0	In 9,966 necropsies
Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936; Congenital Heart Disease, in Nelson's Loose-Leaf Living Medicine, New York, Thos. Nelson & Sons, 1931, vol. 4, p. 246	2		In 1,000 cases of congenital heart disease

The fact that multiple fenestras occurred with the other cardiac anomalies suggests a similar congenital origin for the former. Foxe,[2] however, expressed the opinion that the fenestras are not congenital but are fundamentally mechanical in origin, produced by the constant strain of the current. In favor of this hypothesis is the increasing frequency of fenestrations with advancing age. In a series of 300 autopsies Foxe noted that one or more fenestrations of the semilunar valves occurred in 82 per cent of the subjects and that the frequency of involvement of the aortic and pulmonary valves is equal.

The clinical significance of the supernumerary cusp lies only in its tendency to become the seat of endocarditis.

2. Foxe, A. N.: Am. J. Path. 5:179, 1929.

GRANULOMA VENEREUM SIMULATING CARCINOMA

Report of a Case with Diagnostic and Therapeutic Complications

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ST. LOUIS

IN THE surgical and dermatologic departments of the Barnard Free Skin and Cancer Hospital the incidence of diagnosed granuloma venereum (granuloma inguinale) has been extremely low, with 8 cases diagnosed in approximately 75,000 admissions in twenty-three years. In an institution where the clinicians are cancer conscious it is readily understandable that a lesion of long standing (several months or longer) presenting remarkable hyperplasia, associated with palpable regional lymph nodes and following trauma should be diagnosed clinically as carcinoma. It is not surprising, therefore, that when a patient presented himself at the surgical clinic with a lesion on the penis with such a history, cancer was suspected. Routine biopsy of tissue did not confirm the clinical impression completely but induced the pathologist to lean toward a diagnosis of carcinoma and to request specimens for further biopsies.

In several papers, Pund and Greenblatt[1] have described the histopathologic aspects of granuloma venereum, emphasizing certain criteria by which the specific lesion of this disease may be recognized. In presenting the histologic features they pointed out[1a] that:

> The floor of the ulcer is composed of granulation tissue, which sometimes becomes exuberant. The marginal epithelium usually shows pronounced proliferative changes. The epithelial hyperplasia may be so great and the granulation tissue reaction in the papillae so massive that the rete pegs become elongated and the resultant relationship so distorted that islands of keratinized epidermis are frequently found deep in the section. These give the appearance of epithelial pearl formation and may simulate early carcinoma.

In another publication,[1c] in their conclusion, they stated that "a casual relationship with cancer is suggested."

In the case reported here the granuloma had the characteristics described by Pund and Greenblatt except for a paucity of the "pathognomonic large mononuclear cells." The resemblance to carcinoma was apparent both clinically and histologically. In addition, there were the sinuses with constant discharge, the gram-negative bacilli of the colon group found in the edematous epithelium and in culture and, in tissue and in culture, a gram-negative bacterium suggestive of Bacterium

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1. Pund, E. R., and Greenblatt, R. B.: (*a*) Arch. Path. **23**:224, 1937; (*b*) J. A. M. A. **108**:1401, 1937; (*c*) Am. J. Syph. & Ven. Dis. **22**:495, 1938.

actinomycetum comitans. The presence of the latter organism suggested an association of actinomycosis. A search for Antinomyces resulted in the finding of the typical Donovan bodies, establishing the diagnosis of granuloma venereum. Because of the various complications, the following case is reported.

REPORT OF CASE

HISTORY.—C. D. G., a white man aged 57, a Missouri farmer, married, presented himself, Oct. 1, 1945, at the surgical clinic of the Barnard Free Skin and Cancer Hospital complaining of a "growth on the penis" of six or seven months' duration. His mother had died at the age of 56 years of cancer of the stomach. The father had died at the age of 58 years of diabetes. Two maternal uncles had had cancer, one cancer of the eye and the other cancer of the rectum. The rest of the family history was of no great importance. The patient stated that he had experienced no serious illnesses and no operations. He had had the usual childhood diseases. Three ribs had been fractured in an automobile accident but had since healed. His general health had always been good except for an episode twenty years previously when he had had blood poisoning and adenopathy had been noted in the right axillary region. The patient denied having had any venereal diseases. On questioning, however, he admitted that he had a "wart" on the end of the penis, which had been there for twenty years.

His illness apparently had its beginning in February 1945 when he accidentally ran a straw hook into his penis. Several days later the penis became swollen, red and painful and drained pus. Two weeks later another blow to the penis caused more drainage. This cleared up, but in May the penis again became swollen, and the swelling was accompanied by tenderness. In June he consulted an osteopath, who incised the lesion. A short time later the osteopath again incised the lesion and inserted drains. This treatment was repeated on several occasions, but the lesion failed to improve. He still had two draining sinuses, and the penis was swollen and tender. He had had "kernels" in the groin shortly before coming to the clinic, but these had disappeared. In the past three months he had lost 25 pounds (11.3 Kg.). Blood was drawn for a Kahn test. Because the lesion clinically suggested carcinoma, tissue was removed for biopsy, and the patient was scheduled for admission to the hospital for penisectomy and bilateral dissection of inguinal lymph nodes, depending on the pathologist's report.

Examination.—When admitted to the hospital, October 11, the patient, a tall, lean man, appeared alert, cooperative and neither acutely nor chronically ill. Except for a few shotty lymph nodes in the inguinal region and for the lesion on the penis, the general examination revealed no abnormalities or unusual signs or symptoms. The penis was not circumcised. On the distal half was a large granulomatous lesion, which was swollen, red and acutely tender. On the left side of the shaft was a fungating, necrotic, ulcerating lesion, measuring 5 by 5 cm. The edge of the lesion appeared rolled and somewhat pearly. On the right side of the shaft were similar smaller ulcers, but these were not raised to the same height. On the dorsum of the penis were two punched-out lesions, measuring approximately 0.5 cm. in diameter. The lesions were traversed by sinuses which exuded a foul discharge consisting of a slimy transparent substance, pus and blood. The urethral meatus was not involved. On the glans there was a small warty excrescence, which measured 0.5 cm. in diameter. In the right and left inguinal regions there were felt freely movable lymph nodes, which were not much larger than those usually felt. The blood pressure was 140 systolic and 80 diastolic; the pulse

rate was 88 and the respiration rate 20. The clinical impression was carcinoma of the penis, possibly with metastases to lymph nodes.

Urinalysis gave negative results. The results of the Kahn test and the Frei test were negative. The blood count showed 4,410,000 red cells and 10,750 white cells; the hemoglobin content was 84 per cent. There were 64 polymorphonuclear neutrophils, 22 lymphocytes, 14 stab forms and no eosinophils, basophils or mononuclears per hundred white blood cells.

Microscopically, sections of tissue taken for biopsy showed pronounced hyperplasia of the prickle cells, which, however, seemed to be fairly orderly. At one site it was difficult to be sure whether a mass of prickle cells had actually invaded the dermis or whether this mass of cells merely represented a clubbed end of an extended rete peg. There was a great deal of chronic inflammation in the dermis. In view of the evident difficulty of reaching a dogmatic conclusion, additional tissue was requested for biopsy. Accordingly, October 17, tissue was excised from the penile lesion. Grossly, this specimen was irregular and verrucous, and on cut section the epidermis appeared thickened, but there was no evidence of invasion of the underlying dermis. The microscopic observations were similar to those made in sections of the first specimen. Cultures for fungi showed none.

Fig. 1.—Granuloma venereum of the penis.

Course.—The patient was discharged October 31, so that he could attend to his affairs at home. He was readmitted to the hospital November 5. There was no change in the penile lesion. Clinically, the resemblance of the lesion to carcinoma was still being considered. Consequently, November 9, more tissue was excised for biopsy, one specimen from the shaft of the penis and the other from the glans. Microscopically, sections from the first specimen showed pronounced epithelial hyperplasia and well defined chronic inflammation. The tissue from the glans appeared similar to that from the shaft and showed somewhat more variation in the shape of the staining reaction of the cells. The pathologist's report, therefore, was epithelial hyperplasia and chronic inflammation. The lesions were treated with wet packs, but there was no change in their appearance. The patient was discharged Nov. 16 and readmitted Jan. 4, 1946 for further study.

In the interval between his discharge, November 16, and his readmission, January 4, the patient had been working and felt well except for severe pain in the penis. He had gained weight and had had no illness other than the pain noted. On admission it was noticed that the penile growth had increased in size; this increase was most pronounced on the ventral region. The penis had been very tender and the growth very friable; however, the pain had become less acute, and the odor and the discharge were lessened. The larger mass appeared sclerosed.

The blood showed no changes other than those observed on the first admission to the hospital.

On January 19, cultures were made from sinus curettings and from the purulent discharge. An organism identified as B. actinomycetum comitans and also a growth of a gram-negative bacillus considered to be a member of the colon group were obtained on artificial mediums. Because of the inflammation, swelling and pain, the patient was given penicillin intramuscularly, 30,000 Oxford units every four hours. This dosage was followed the next day with 30,000 units every three hours until a total of 2,100,000 units had been administered. The acuteness, drainage, redness and pain subsided somewhat, but there was no apparent change in the size of the lesion.

On the basis of the finding of gram-negative bacteria in cultures, the patient was started on parachlorophenol (0.25 per cent in saline solution) according to the work of Meleney.[2] The solution was injected through a suitable bulb syringe into the sinuses. The patient complained of a burning sensation and then he felt relieved. During the afternoon and evening, blood started oozing out from the lesion, and the patient lost a considerable amount of blood. The use of parachlorophenol was discontinued, and dressings soaked in a solution of potassium permanganate were applied. Eight days later, February 19, parachlorophenol was again used, but only as a solution in which dressings were soaked for application. This therapy had no effect on the lesion and was accordingly discontinued after a few days.

The finding of B. actinomycetum comitans suggested the possibility of actinomycosis, since the statement has been made in the literature that this organism invariably is a concomitant finding in actinomycosis and "it has never been described in the absence of actinomycosis."[3] Consequently, the administration of a saturated solution of potassium iodide was begun, the dosage starting with 5 drops three times a day and increasing by 2 drops daily. By the time the patient was receiving 19 drops three times daily, some improvement of the condition of the lesion was noticed. While sections of tissue stained by the Gram-Weigert method were being searched for Actinomyces, the characteristic gram-negative Donovan bodies were found. The use of the iodide was discontinued and stibophen ("fuadin" [sodium antimony III biscatechol—2, 4 disulfonate]) was substituted, 1.5 cc. being injected intramuscularly the first day, 3.5 cc. the second day and 5 cc. daily thereafter until a total of 50 cc. had been injected. No immediate change was noted. Because of the continued foul odor, application of dressings soaked with a solution of potassium permanganate was started. March 26, the blood showed 3,950,000 red cells, 8,400 white cells and 78 per cent hemoglobin. There were 60 polymorphonuclear neutrophils, 29 lymphocytes, 8 stab forms, 1 eosinophil and 2 mononuclears per hundred white blood cells. The patient was discharged the following day.

May 30, the patient returned to the hospital, and a second course of stibophen therapy was started, beginning with an intramuscular injection of 2 cc. He was readmitted to the hospital the next day and was given an injection of 5 cc., and he continued to receive this drug, 5 cc. every two days, until a total dose of 40 cc. had been given.

June 1, smears were made from the lesions, which had increased in size, involving the entire penis to the base, with multiple sinuses developing and ap-

2. Meleney, F. L.; Johnson, B. A.; Pulaski, E. J., and Colonna, F.: J. A. M. A. **130**:121, 1946.

3. Klaber, R.: Brit. J. Dermat. **46**:12, 1934.

parently profuse secondary infection. Donovan bodies were not found in stained smears, but large numbers of spirochetes were observed. The spirochetes were considered to be of the Vincent type, although fusiform bacilli were not noticed. Because of the secondary infection, dressings soaked in glycerine and hydrogen peroxide (equal parts) were applied. In addition, 0.06 cc. of oxophenarsine hydrochloride ("mapharsen") was given on June 1 and on June 4. The use of the aforementioned dressings was discontinued temporarily, and others soaked with a solution of tyrothricin (60 cc. of water to which tyrothricin was added to a concentration of 2 per cent) were applied. As a palliative measure, 500 roentgens of high voltage roentgen radiation (filter 0.9 half-value layer; 200 kilovolts; 18 milliamperes; focal skin distance, 50 cm.) was administered through two ports. There was some temporary reduction in the size of the mass. It was felt that no further medical treatment was justified, and the patient was recommended to the surgical department.

June 21, with the patient under spinal anesthesia, the penis was amputated. Two days after the operation, the scrotal sac appeared swollen, tense and tender, and there was bilateral epidymitis. These inflammations were treated with scrotal support, ice packs and penicillin. Dressings soaked in a solution of aluminum acetate were started. A slough formed and gradually separated. On July 15, the operative wound began to heal by secondary intention. The patient left the hospital and returned two weeks later. He stated that he felt fine and had no trouble urinating. The wound showed remarkable healing.

PATHOLOGIC OBSERVATIONS

The first specimen taken for microscopic study, Oct. 1, 1945, was from the lesion of the shaft of the penis. Sections of tissue showed pronounced hyperplasia of the epidermis with pseudoepitheliomatous branching into the dermis. Several masses of epithelial cells were seen in the dermis, but it was difficult to tell whether these were isolated groups or merely the clubbed ends of rete pegs. The epidermis was noticeably edematous, showing both intercellular and intracellular edema, with pronounced intercellular bridges in certain areas. In some sections there was evidence of necrosis in the epidermis, indicating an ulcerous process. An outstanding feature was the extensive parakeratosis with vacuolation of the cells. The prickle cells were extremely swollen, and a probable fluid uptake was indicated by the clear spaces within the cells. Present among the edematous prickle cells and invading many of them were numerous gram-negative bacilli, both small and large forms. The large forms in some instances had taken on the peculiar star-shaped appearance (fig. 2*A*).

The dermis was greatly thickened by a chronic inflammatory process. The infiltrate, composed chiefly of plasma cells and lymphocytes, was most concentrated in the upper third of the dermis in close contact with the rete mucosum. In some areas the rete showed the effect of the inflammatory response, as evidenced by the distortion and swelling of the prickle cells. Throughout the dermis but most numerous in the areas of intense infiltration and in the interpapillary spaces were small capillaries, engorging red blood cells. Small foci of polymorphonuclear leukocytes were seen in the dermis and scattered throughout the epidermis. The pathologist considered the diagnosis of questionable early squamous cell carcinoma.

A second specimen was taken for biopsy, October 17, from the shaft of the penis. On section, the epidermis was greatly thickened, but there was no gross evidence of invasion of the underlying dermis. Microscopically, the section appeared to be that of a papillary growth with a good deal of epithelial hyperplasia. A few

rather large islets of epithelial cells could be seen deep in the dermis. There was no apparent increase in mitotic activity, and the arrangement of the cells in these deep masses was orderly. In general, the picture did not contribute anything additional to what could be noted in the first biopsy specimen. This was considered by the pathologist as papilloma with possible early malignant change.

November 9, two more specimens were taken for microscopic examination. One was from the glans penis and the other from the shaft of the penis. The specimen from the shaft was firm in consistency and grayish. Microscopically, sections of the glans showed a picture the same as that seen in the first biopsy specimen except, perhaps, that there appeared to be somewhat more variation in the shape and the staining reaction of the cells. Sections made of the specimen from the shaft also showed extensive epithelial hyperplasia and well defined zones of chronic inflammation. Scattered throughout the inflammatory dermis were aggre-

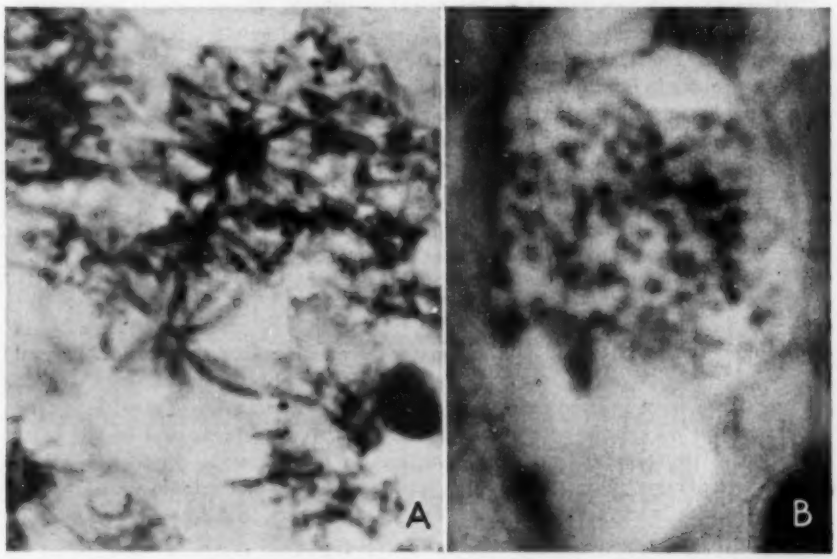

Fig. 2.—*A*, photomicrograph of the superficial layer of the lesion showing gram-negative bacilli, some arranged in rosette formation. Gram-Weigert stain; × 1950.

B, Donovan bodies in a large histiocyte. Gram-Weigert stain; × 3650.

gates of cells, chiefly lymphocytes and plasma cells, some having assumed the characteristics of pseudotubercles. Surrounding these pseudotubercles there was a zone of edematous connective tissue, within which could be seen large vacuolated cells. When sections were stained by the Gram-Weigert method, typical Donovan bodies were found within some of these large histiocytes (fig. 2*B*). The pathologist's report on the sections stained with hematoxylin and eosin was epithelial hyperplasia and chronic inflammation.

June 21, 1946, the amputated penis was received in the laboratory. The entire glans and shaft were distorted by a fungating, ulcerated, necrotic mass. The specimen measured 10 cm. in length and 6 cm. in diameter. There were numerous sinuses in the mass. On section the entire shaft appeared destroyed by the lesion, which surrounded the wall of the urethra and extended through the muscle layer.

A block was taken from the posterior surface of the penis involving a papillary portion of the mass. On the right lateral surface of the shaft there was a fungating, ulcerated area, 5 cm. in diameter, bounded by hyperplastic skin. On section the skin of this area appeared hyperplastic and papilliform in its arrangement. The hyperplasia, however, was sharply delimited. A third block was taken from the edge of this area. A fourth block was taken from the glans.

Microscopic examination of sections of the first block revealed a hyperplastic and edematous epithelium. In one focus there was an abscess, and the epithelium covering this area was completely eroded. The lymph spaces and blood vessels in the underlying connective tissue were dilated, and the blood vessels were engorged. There was a dense cellular infiltrate throughout the connective tissue which was made up of large numbers of plasma cells, lymphocytes and polymorphonuclear leukocytes. Some large histiocytes were also present, and in some of these cells Donovan bodies were found.

The picture of sections of the second block was essentially similar to that described for the sections of the first block except that the epithelium was more papillary in its arrangement and was intact throughout. The infiltrate in the connective tissue was less dense than that seen in the first block. Sections of the third and fourth blocks showed pictures essentially the same as that described for the sections of the second block.

The pathologist's diagnosis was granuloma venereum (granuloma inguinale).

It was apparent, therefore, that until the Donovan bodies were demonstrated in tissue, the pathologist inclined toward a questionable diagnosis of carcinoma. In general, from a histologic, clinical and therapeutic standpoint such a diagnosis may have had its justifications.

SUMMARY

A case of granuloma venereum (granuloma inguinate) of the penis of a 57 year old white man is presented in which the lesion clinically simulated carcinoma. Sections of tissue revealed extensive hyperplasia of the rete mucosum, with the proliferated tissue extending into the cutis, and chronic inflammation, the whole resembling carcinoma. Several subsequent biopsies, however, failed to establish a definite diagnosis of carcinoma. Careful observation of the sections revealed a reaction which was characteristic of granuloma venereum. This was confirmed by the finding of the typical Donovan bodies. The finding of gram-negative bacilli of the colon type and of the type described as Bacterium actinomycetum comitans confused the diagnosis. To complicate the picture further, the lesibn was resistant to all forms of therapy used, so that, as a final resort, penisectomy was performed. The wound showed good healing, and the patient was able to urinate without trouble.

Notes and News

Appointments, Etc. —The United States Senate on April 26 confirmed the appointment of Col. Raymond O. Dart, director, Army Institute of Pathology, as a permanent brigadier general in the Medical Corps of the Army. General Dart, a native of Kansas, is a graduate of the University of Kansas and of Rush Medical College; he interned at the Presbyterian Hospital, Chicago, and since entering the Army in 1917 has spent most of his service in pathology. He served in Europe in World War I and for a time was commandant of the 105th General Hospital in World War II, surgeon of the Brisbane, Australia, base and New Guinea advanced section and deputy chief surgeon in the Pacific Area. He has been awarded the Legion of Merit and the Bronze Star.

J. T. Syverton, professor of microbiology in the State University of Louisiana, has been appointed professor and head of the department of bacteriology and immunology of the University of Minnesota Medical School.

G. John Buddingh, formerly professor of bacteriology in the school of medicine of Vanderbilt University, has been appointed professor of microbiology in the school of medicine of the State University of Louisiana, New Orleans.

Russell V. Milliser, formerly assistant professor of pathology, Ohio State University Medical School, has been appointed associate professor of pathology in the Chicago Medical School.

Charles S. Cameron, New York, has been appointed medical and scientific director of the American Cancer Society. He has served as assistant, acting and associate medical and scientific director, and director of the service department. He is clinical assistant surgeon at Memorial Hospital, New York.

G. Selin has been appointed assistant pathologist at the Hospital for Joint Diseases, replacing L. Lichtenstein. Dr. Selin was formerly instructor in pathology at the New York Medical College.

L. W. Slanetz is now professor of bacteriology and head of the department of bacteriology at the University of Kansas School of Medicine, Lawrence-Kansas City, succeeding N. Sherwood, head of the department for thirty years, who has retired from administrative duties but will continue to teach.

Awards. —On Dec. 10, 1947, Gerhard Domagk received in Stockholm, Sweden, the Nobel Prize for the discovery of the therapeutic effects of the sulfonamide drugs. Under the Nazi régime he had been forbidden to accept that award.

The George M. Kober Medal of the Association of American Physicians has been awarded to W. T. Longcope, Lee, Mass., formerly professor of medicine at the Johns Hopkins University, for the contributions he has made to medicine and to medical education.

The Association for the Study of Internal Secretions has given the Squibb Award for 1948 to Fuller Albright, Harvard Medical School, for his work on the functions of hormones. The 1948 Ciba Award has been given to Carl G. Heller, University of Oregon College of Medicine, for work on human disorders of reproduction.

Death.—C. Bonne, professor of pathology at the School of Medicine, Batavia, Java, died recently in Amsterdam, Holland. An authority and frequent contributor to the literature dealing with the problem of racial cancer and other diseases, Professor Boone spent the occupation years in Australia, returning to Java after the war.

Society News.—The American College of Physicians will conduct its thirtieth annual session in New York, March 28 through April 1, 1949.

The American Veterinary Association met in the Palace Hotel, San Francisco, August 16 to 19.

The Biologic Photographic Association will meet in Houston Hall, University of Pennsylvania, next September 8 to 10. There will be an exhibit and demonstrations. For information write to the secretary, Magee Hospital, Pittsburgh 13.

Fellowships.—Clinical fellowships in the diagnosis and treatment of cancer have been established by the American Cancer Society, Inc., for young physicians and may be secured through hospitals designated by the society that offer postgraduate training in cancerology, surgery, obstetrics and gynecology, orthopedic surgery, otolaryngology, urology, radiology, pathology and internal medicine. The fellowships, renewable for three years, carry a stipend of $2,400 to $3,600 per year.

The American College of Physicians announces that a limited number of fellowships in medicine will be available from July 1, 1949 to June 30, 1950. These fellowships are designed to provide an opportunity for research training either in the basic medical sciences or in these sciences as applied to clinical investigation. They are for the benefit of physicians in the early stages of their preparation for a teaching and investigative career in internal medicine. Assurance must be provided that the applicant will be acceptable in the laboratory or the clinic of his choice and that he will be provided with the facilities necessary for the proper pursuit of his work. The stipend will be from $2,200 to $3,200. Application forms will be supplied by the American College of Physicians, 4200 Pine Street, Philadelphia 4, and must be submitted in duplicate not later than Nov. 1, 1948. Announcement of the awards will be made as promptly as is possible.

Electron Microscopists.—The Electron Microscope Society of America has on its roll the names of every active electron microscopist in the country. Employers seeking scientists or technicians to work in this field may use its placement service. Inquiries should be directed to the secretary, Dr. C. J. Burton, American Cyanamid Company, Stamford, Conn.

Biologic Steroids.—A symposium on biologic steroids will be held at the University of Wisconsin on September 6, 7 and 8 next. Persons who are interested in attending the entire symposium can secure reservation for room and board by writing to Dr. H. Lardy, University of Wisconsin, Madison 6.

Appointments, Etc.—Maurice Lev, of the department of pathology of Michael Reese Hospital, has been appointed assistant professor of pathology at the University of Illinois College of Medicine, Chicago.

Announcement has been made of the retirement of George M. Smith, professor of anatomy (research) in Yale University, medical director of the Anna Fuller Fund and of the Jane Coffin Childs Memorial Fund for Medical Research, and until recently executive director of the National Advisory Cancer Council.

Award.—Paul R. Cannon, chairman of the department of pathology of the University of Chicago, has been given the Ward Burdick Gold Medal of the American Society of Clinical Pathologists and the gold medal award of the Philadelphia Pathological Society.

Society News.—The American Association of Pathologists and Bacteriologists announces that its forthcoming annual meeting will be held in Boston, April 15 to 16, 1949. Further information regarding the meeting may be obtained from the Secretary, H. T. Karsner, 2085 Adelbert Road, Cleveland.

The Fifth International Congress for Comparative Pathology will be held at Istanbul, May 17 to 20, 1949, and will be under the chairmanship of Akil Moukhtar Ozden. Papers on problems of medical, veterinary or plant pathology may be submitted to Dr. Louis Grollet, secrétaire général, Comité International Permanent des Congrès de Pathologie Comparée, 7 rue Gustave Nadaud, Paris 16e, France.

Microbiological Institute in the United States Public Health Service.—According to *Science,* this institute has been established as part of an extensive realinement of the medical research program of the National Institutes of Health. Together with the Experimental Biology and Medicine Institute, established last December, the new unit will deal with research in such diseases as malaria, poliomyelitis, typhus and the common cold, as well as basic research studies in physics, chemistry, nutrition, metabolism and pathology. The new institute will also do work in the establishment of standards insuring the safety, the purity and the potency of serums, antitoxins and vaccines for human use. Victor H. Haas will be the director of the new organization.

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Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.